NITROGEN FERTILIZATION AND NITRATE ACCUMULATION IN SOME HAWAIIAN PLANTS AND SOILS

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INTRODUCTION

Nitrate - N is one of the major nutrient forms of inorganic N present in soils. It plays a very important role in the nutrition of most plants and it often is a limiting factor in plant growth. In most cases the nitrate absorbed by plants is rapidly assimilated and thus its concentration in plant tissue is very low. However, nitrate has been observed to accumulate in abnormally high concentrations in plant tissue when certain conditions prevail. An accumulation of nitrate in plant tissue is not injurious to the plant; in fact, in some crops the nitrate content has been shown to be positively correlated with yield, and tissue testing of these crops has been advocated as a guide to optimum fertilization. The considerable interest shown to nitrate accumulation in plants today is because of its potential hazard to the health of humans and animals.

When a material high in nitrate is ingested, the nitrate will be reduced to nitrite. The nitrite is readily absorbed in the blood stream and reacts with the hemoglobin in the blood to form methemoglobin, which can be lethal if present in high amounts. This physiological disorder is referred to as methemoglobinemia. In livestock when the methemoglobin level is not high enough to produce lethal effects, sub-lethal effects, such as low milk production, abortion, reduction in Vitamin A and poor growth rate, can occur. In silos, gaseous decomposition products of plant material high in nitrate can cause injury or death if inhaled.

In past decades N fertilizers have been liberally used in crop production, especially in forages and vegetables. There also has been a large increase in livestock production. Disposal of animal waste is an increasing problem. Animal waste is generally known to have nutrients that can be utilized effectively by plants and also can produce some physical changes in the soil desirable for plant growth. Recognition of the beneficial effects of manure application to crop production has led to the suggestion that the final solution of the animal waste disposal problem is to see that the waste all goes back to the land for reutilization. Due to lacks of adequate storage and transportation, and other limitations of manure application, increasing tonnages of manure are being applied to land close to the source. This practice has no doubt increased the efficiency of crop production to some extent. However, questions have been raised concerning what effects these practices are having on nitrate pollution and other environmental problems.

Considerable amount of work has been done in this area. Most of the work, however, has been centered in places where serious economic losses due to nitrate poisoning have occurred. Study of this problem in the tropics is lacking. Although in Hawaii the case of animal loss through nitrate poisoning has not been fully documented, there have been some incidents in which animal losses have been suspected to be caused by nitrate poisoning. There also have been changes in agriculture practices in the tropics that may introduce favorable conditions for nitrate accumulation in plants. Thus, these situations call for more nitrate research to be extended to the tropics.

Under normal conditions, most plants have a low nitrate content; however, there are several species which are known to be naturally high nitrate accumulators. These plants should be identified so that their inclusion in animal feed can be avoided. This information would be useful for livestock production. In most crop production programs, especially with forage and vegetable crops, maximum vegetative yield is often the main objective and N is often applied in large amounts, together with other fertilizer elements. The possibility of toxic amounts of nitrate accumulating in these crops is an increasing problem. Thus, an understanding of the effect of increasing soil - N on nitrate accumulation would be very useful so that accumulation of excessive nitrate in plants can be avoided. The specific objectives of this study were as follow:

1. To survey the nitrate - N content of some common weeds and forage crops in Hawaii.
2. To study the effects of inorganic N fertilization on nitrate accumulation in plants.
3. To study the effects of manure application on nitrate accumulation in plants.

REVIEW OF LITERATURE

A. Nitrate Poisoning

One of the earliest reports of what might have been nitrate poisoning in animals was written by Mayo in 1895 (Mayo, 1895). He reported that cattle had died after feeding on corn stalks; the nitrate content in the corn plant was found to be as high as 11.5% (expressed as KNO_3) and KNO_3 crystals were found in the corn tissue. At that time, Mayo attributed the toxicity to potassium rather than to nitrate. Other reports at that time also tended to associate high KNO_3 in feed material with animal toxicity. It was not until 1939 when Bradley *et al*. (1940) offered evidences that toxicity was actually caused by nitrate. Later, several cases of poisoning in cattle, sheep, pigs, and even humans (infants), were traced to the consumption of material high in nitrate (Buchman, 1968; Diven, 1964; London, 1967; Phillips, 1971; Marrett and Sunde, 1968).

The so-called "nitrate poisoning" is actually "nitrite poisoning". Nitrate ion *per se* is relatively non-toxic to mammals. It was shown in dogs that the nitrate ion was easily absorbed and readily excreted by the kidney (Greene and Hiatt, 1954). Under certain circumstances, however, nitrate can be reduced to nitrite by microbial action in the gastro-intestinal tract. It was proposed by Lewis (1951) that nitrate is reduced to nitrite and finally to ammonia in the digestive tract. If a high amount of nitrate is being ingested, a toxic amount of nitrite can be accumulated. Ruminant animals would favor more nitrite production than non-ruminants because the feed material is kept longer in the rumen, allowing more microbial activity to take place. The nitrite is then readily absorbed into the blood stream and reacts with hemoglobin to form methemoglobin. There is evidence that methemoglobin in the blood alters the affinity of oxygen for hemoglobin in a manner analagous to that of CO tending to make the release of oxygen from the blood to the tissues somewhat more difficult (Bodansky, 1951). This physiological disorder is referred to as "methemoglobinemia". In infants it is also called the "blue babies disease" and in animals it is sometimes called the "corn stalk poisoning" or "oat hay poisoning" disease. If the methemoglobin content reaches a toxic level, the animals may die.

In silos, forage high in nitrate may produce poisonous nitrogen oxide gas. This gas can be lethal if inhaled. Several cases of silo gas poisoning in man has been reported (Wright and Davidson, 1964).

Besides the acute deadly effect, nitrate poisoning has been claimed to produce chronic effects, such as low milk production, abortion, reduction in vitamin A and poor growth (Wright and Davidson, 1964; Jones, 1966; Davidson, 1965; Hoar, 1968; Sell and Roberts, 1963). However, these claims have not been well-documented. There are several reports that show that nitrate does not affect growth rate and milk production (Davidson, 1965; Jones, 1966). The growth rate is reduced only in instances where the presence of nitrate reduces feed consumption. There are evidences that nitrate can cause abortion in pregnant animals (Davidson, 1965). In lambs nitrate reduces both the plasma and the liver vitamin A; a similar trend is also observed in chicks (Hoar, 1968; Sell and Roberts, 1963).

There is a lack of agreement as to what constitutes a toxic dose for livestock. The confusion arises from the fact that investigators have expressed their findings in different ways; moreover, the nitrate is administered by different procedures and animals of different ages and nutritional status have been used. In dairy cattle, Simon (1959) suggested that 45 mg of NO_3-N per kg of body weight would result in the death of animals. Bradley et al. (1940) proposed that 74 mg of NO_3-N per kg of body weight would be the LD_{50} (the dosage at which 50% of the animals would die). Several other workers have documented different values as the toxic dose. In sheep it ranges from 83 to 175 mg of NO_3-N/kg of body weight; in dairy cows it is between 45 - 56 mg of NO_3-N/kg body weight; in cattle it is 62 to 226 mg of NO_3-N/kg of body weight; in dogs it is 570 mg of NO_3-N/kg of body weight; and in rats it is 800 mg of NO_3-N/kg body weight (Nitrate Accumulation Committee, 1972).

Several investigators have attempted to establish a value of NO_3-N content in forage which would be regarded as toxic to animals. Many workers tend to regard 0.15 to 0.2% of NO_3-N in the forage (on the dry-weight basis) as the "safe" level (Emerick, 1963; Gillingham, 1969; Ryan, 1972; Bradley, 1940). A lower value of 700 ppm (0.07%) of NO_3-N was also encountered (Garner, 1958). Hanway and Englehorn (1958), however, regarded the safe level to be below 2.0% of nitrate.

B. Nitrate Absorption and Assimilation in Plants

In most plant species, the absorbed nitrate is readily assimilated to protein and other nitrogeneous products. Under normal conditions, the rates of nitrate assimilation and absorption are well-balanced so that a high accumulation of nitrate is not possible. However, if the equilibrium is interferred with, nitrate accumulation may result.

It follows that the rates of assimilation and absorption influence the amount of nitrate accumulated.

Ion uptake in plants can occur either through passive or active absorption. Passive absorption of an ion is a physical and non-metabolic process, while active absorption is a metabolic process and requires energy. It has also been known that an ion can be absorbed through ion exchange (Graham and Albrecht, 1943). Absorption of ion by passive absorption and ion exchange is relatively unimportant, especially in an ion uptake. Active ion absorption accounts for the major portion of anion absorbed.

The rate of nitrate uptake has been known to be affected by several factors. pH has been known to affect nitrate absorption in roots; however, there is no complete agreement on how it exterts its influence. Honert _et al_. (1955) found that nitrate uptake was reduced with an increase in pH. In a more recent work, Fried _et al_. (1965) found a similar trend in rice roots. The influence of other anions on the uptake of nitrate has been recognized; they either stimulate, suppress, or have no effect on nitrate uptake. The presence of chloride tends to reduce nitrate uptake due to its antagonistic effect (Bassioni, 1971; Fried, 1965). On the other hand, $H_2PO_4^-$, HPO_4^{-2} and SO_4^{-2} ions have been reported to stimulate nitrate uptake up to a certain concentration, the order of stimulation being $HPO_4^{-2} > SO_4^{-2} > H_2PO_4^-$. This then is followed by a depressing effect in the order of $SO_4^{-2} > HPO_4^{-2} > H_2PO_4^-$ (Bassioni, 1971).

The rate of nitrate uptake in roots is also known to be affected by the valence and concentration of the associated cations. In Bassioni's work (1973), the effect of cations of different valences on nitrate uptake was studied. The cations compared were K^+, Ca^{+2}, Mg^{+2}, La^{+3}, Al^{+3}, NH_4^+ and Th^{+4}. It was found that NH_4^+ caused a slight reduction, while the other cations stimulated absorption with the order of stimulation being $K^+ < Ca^{+2} < Mg^{+2} < La^{+3} < Al^{+3} < Th^{+4}$, i.e. the higher the valence of the cation, the greater was its stimulation. Bassioni (1973) further reported that the concentration of cations also affects nitrate uptake. Low temperature has also been associated with high nitrate absorption suggesting that it is a metabolic process (Honert, 1955).

Reduction of nitrate involves the transfer of several electrons; thus, a large amount of energy is required. Nitrate reduction has long been recognized as an enzymatic process. Much of the earlier work in this field was reviewed by Kessler (1964) and Beevers and Hageman (1969). From these reviews the following overall nitrate reduction scheme in plants was proposed:

$$HNO_3 \rightarrow HNO_2 \rightarrow (HNO)_2 \rightarrow NH_2OH \rightarrow NH_3$$

with nitrite, hyponitrite and hydroxylamine as intermediates.

At least two enzymes have been known to be involved in the reduction process. The enzyme catalyzing the first step of the reaction is nitrate reductase and was first obtained by Evan and Nason (1953). This enzyme contains Fe and Mo, and it receives electrons from NADH or NADPH. During the nitrate reduction process, the Mo serves as an electron carrier. A similar nitrate reductase has been found in higher plants (Candela, 1957; Evan and Nason, 1953; Evan, 1954; Spencer, 1959).

Another enzyme involved is nitrite reductase; it was isolated from fungus (Medina and Nicholas, 1957; Nicholas, 1960) and also from higher plants (Rousos and Nason, 1960). This enzyme has two metal components, Cu^{+2} and Fe^{+3} (Nicholas, 1960). Cu^{+2} seems to act by transferring electrons from flavin to nitrite, whereas the role of Fe^{+3} is still unknown. There seems to be a variation in opinion on the source of energy involved in the reduction. It has been suggested that it is either from carbohydrate respiration or photosynthesis; another proposal is that both sources are involved (Salisbury and Ross, 1969).

C. Factors Influencing Nitrate Accumulation in Plants

Several factors have been known to influence nitrate concentration in plant tissue and they have been reviewed in detail by Wright and Davidson (1964). These factors are either associated with the plant directly, such as, taxonomic unit, part of the plant, stage of maturity, or with the environment of the plant, such as, soil nitrogen, soil moisture, light, temperature, soil type, herbicide and other nutrients. In general, those factors that increase nitrate uptake and reduce nitrate assimilation would favor high nitrate accumulation in plants.

1. Plant Associated Factors

a. Taxonomical difference

Nitrate accumulation is known to differ among plant species and in some cases even among varieties of similar species (Hanway and Englehorn, 1958; Lovelace, 1968; Gilbert, 1946; Hylton, 1970; Murphy, 1967). This difference is attributed to variation in uptake and assimilation of nitrate. However, under appropriate conditions, nitrate accumulation is possible even in plants considered to be low nitrate accumulators (Kretschmer, 1958). According to Crawford et al. (1961), closely related species do not differ significantly.

Several workers conducted surveys on cultivated plants and weeds in their abilities to accumulate nitrate (Hanway and Englehorn, 1958; Gilbert, 1946). These reports indicated that sorghum, oats, millet, sudangrass and several other crops are high nitrate accumulators. Several species of weeds are known to be high nitrate accumulators, too; these are: Amaranthus spp, Russian thistle, fireball and lambsquarter (Gilbert, 1946; Sund, 1957). It has been suggested that plants that have a high stem to leaf ratio are high nitrate accumulators (Emerick, 1963).

Under comparable conditions, different varieties of the same species of plants have been observed to differ in their abilities to accumulate nitrate. Crawford et al. (1961) suggested that this difference is due to a variation in the nitrate reductase activity. Different varieties of orchardgrass under comparable condition have been observed to differ significantly in their nitrate content (Dotzenko and Henderson, 1964). Other workers (Gul and Kolp, 1960; Hylton, 1970) reported similar trends in barley, bermudagrass, corn, oats and wheat. Hoener and De Turk (1938) suggested that in corn, high protein varieties absorbed and assimilated nitrate faster than the low protein varieties; thus, a higher accumulation was found in the low protein varieties.

b. Part of Plant

Several workers (Hanway and Englehorn, 1958; Whitehead, 1948; Flynn, 1960, and Crawford, 1961) reported that the nitrate concentration in plant tissues varied with the morphological parts. In corn and sorghum, the nitrate concentration if ranked in an increasing order, is as follows: tassel, head, leaves, leaf sheath, and stalk (Whitehead, 1948). Several other workers (Crawford, 1961; Flynn, 1960; Hanway and Englehorn, 1950) working with corn, oats and other grain plants reported a similar trend. In most of these reports the highest concentration of nitrate was found in the stem. It has also been observed that the lower portion of a plant tends to be higher in nitrate than the upper portion (Whitehead, 1948).

In some reports the highest nitrate content was found in other parts of the plants. Bradley (1940) reported that more nitrate was found in the leaves than in the stalk or head in oats. Woo (1919) reported that in pigweed more nitrate is found in the branches than in the main stem. In sugar beet, the leaf blade accumulated as much nitrate as the root (Cantliffe, 1974).

The variation in nitrate concentration with plant parts seems to be associated with the site of nitrate reduction. Low nitrate reduction has also been associated with non-chlorophyllous tissue, which accounts for the high nitrate in the stem, leaf base and lower leaves (Sideris and Young, 1947).

c. Stage of Maturity

In most experiments involving periodic sampling of plants through a cycle of growth, indications are that the nitrate content first rises and then, after reaching a peak at about the pre-flowering stage, declines as the plant matures. This trend have been demonstrated in oats, corn and in several forage crops (Crawford, 1961; Gonske and Keeney, 1969; Murphy and Smith, 1967; Whitehead, 1948; Gul and Kolp, 1960).

Several reasons have been suggested to explain the decline in nitrate content with the advancement of maturity. With maturity, plants usually accumulate more dry matter; this accumulation will alter the composition of the tissue with nitrate content tending to be diluted. It is also suggested that as the plant matures, there is a high demand for N for fruit and seed development (Flynn, 1957). And finally, the N-supplying power of the soil tends to decline as the plant matures, allowing the plant to assimilate the nitrate that has been accumulated. Crawford (1961) found that in sand culture, when the nitrate supply was replenished throughout the growth cycle, the nitrate content of the plants did not differ with age (Crawford, 1961). Several other reports are in agreement with that of Crawford (Stalh and Shive, 1933; Nowakowski, 1961).

2. Environmental Factors

a. Nitrogen Supply

The effect of N supply on the nitrate content in plants has been given greater attention than other factors. It is obvious that nitrate accumulation can occur only if the external medium can supply nitrate at a faster rate than the rate of nitrate assimilation, unless some nitrogenuous compounds in the plant can be oxidized to nitrate.

Experiments on the effect of N on nitrate accumulation are numerous; a few will be cited here. Most of the reports indicate that the nitrate content in plants is positively correlated with the external N supply. This relationship has been demonstrated in corn, oats, sudangrass, sorghum, spinach, sugar beet and several other crops (Flynn, 1957; Hanway and Englehorn, 1958; Gilbert, 1946; Cantliffe and Goodwin, 1974; Vanderlip and Pesek, 1962; Barker and Tucker, 1971).

A majority of the experiments conducted were designed to study the characteristic of the nitrate response curve so that a "safe" level of N application could be predicted. The concentration of nitrate - N in oats increased linearly up to 200 lb. N/A; a still further increase was observed to about 800 lb N/A (Crawford, 1961). Ryan et al. (1972) working with perennial grasses concluded that up to 270 kg N/ha could be applied to forage with no serious adverse effect on nitrate - N level; nitrate - N above 0.15% was considered to be toxic. On the other hand, Murphy and Smith (1957), also working with perennial grasses, reported that N treatment above 112 kg/ha resulted in a nitrate - N concentration above 0.07% nitrate - N, which he considered as an "unsafe" level. A very high value of 3,500 ppm of nitrate - N in the tissue was reported in rye and oats when seven levels of N from 0 to 1440 kg of N/ha were used (Barker and Tucker, 1971). Hanway and Englehorn (1958) reported that an application of 16 T/A of manure doubled the nitrate content of corn stalks for the first- and second-year crops, but when only 8 T/A of manure was applied the nitrate content was doubled only in the first-year crop (Hanway and Englehorn, 1958). On the other hand, Wright and Davidson (1964) claimed that several cases have been known in which N application did not increase nitrate concentration in the plant tissue.

The timing of N fertilization has been shown to affect the nitrate content of many forage crops; an application just before harvesting tends to increase accumulation. Hojjati et al. (1972) reported that in tall fescue, rye and bermudagrass, at comparable N level, plants harvested at less than 30 days after N application had a higher nitrate content than plants harvested at a longer period, while Ryan (1972) claimed that a split application of N fertilizer resulted in a higher accumulation than bulk application. A similar trend was also demonstrated by Crawford (1961); however, he attributed the difference to leaching.

It has been established that most plants can utilize all the four major forms of N for growth: nitrate, nitrite, ammonium and organic N. However, under field conditions a major portion of the N is converted to nitrate by microbial activity. Studies on the effect of different N carriers on nitrate accumulation in plants have shown that the differences are small or lacking altogether (Crawford, 1961; Gilbert, 1946). Cantliffe and Goodwin (1974) found that in sugar beet a substantially higher nitrate content was associated with nitrate fertilizers as opposed to ammonium fertilizers at equivalent N amounts.

b. Effect of Other Nutrients

Recently, studies on the effects of other plant nutrients on nitrate accumulation have received great attention. Relatively more work has been done on K than the other nutrients. Most researchers agree that the uptake of nitrate is enhanced by the presence of a cation, such as K^+ (Hylton, 1970; Lawton and Cook, 1954). There have been many different opinions, however, as to whether K increases nitrate accumulation or nitrate reduction. Nightingale (1930) showed that K^+ was necessary for the synthesis of organic N from nitrate. A similar finding was reported by Lawton (1954).

Other investigations have demonstrated that K^+ did not encourage nitrate reduction in plants. Sideris and Young (1938) found that higher concentrations of K increased nitrate concentration in plants grown in a nitrate solution. In tobacco, a slightly higher nitrate content is found in leaves high in K than in leaves low in K (Peterson and Chester, 1968). Schneider and Clark (1970) worked with pearl millet and sudangrass and reported that those plants which received 672 kg/ha of K had a two-fold increase in nitrate content, while in an earlier report, Smith and Clark (1968), working with similar species, showed that K^+ increased nitrate accumulation only in pearl millet, and nitrate content in sudangrass was not affected. It was reported that the increase in nitrate - N and K concentration was not followed by an increase in the total - N concentration, suggesting that K does not increase nitrate reduction (Smith and Clark, 1968).

The effect of P on nitrate accumulation has not been well established. It was shown by Barker and Tucker (1971) that the concentration of nitrate - N in wheat forage was reduced from 1,300 ppm to 300 ppm with an application of 15 kg/ha of P. In smooth bromegrass, it was found that the application of P also reduced nitrate - N content (Vanderlip, 1970). Other reports (Murphy and Smith, 1967; Smith and Clark, 1968), however, showed that P has little effect on nitrate - N content.

Very little work has been done on the other nutrients. In pearl millet and sudangrass, Ca tends to lower nitrate - N content (Schneider and Clark, 1970). Mg has been shown to have little effect in pearl millet; however, the work of Schneider (1970) indicated that Mg reduced nitrate concentration in plants even more than Ca. In table beet, it was shown that chloride - containing fertilizer reduced nitrate content in plants (Cantliffe, 1974). This was attributed to the antagonistic effect between the two similar anions. Imbalance or deficiency of a micronutrient, such as Fe, Mo, and Mn, would affect nitrate accumulation. Crawford (1961), in his work with microelements, indicated that in sand culture, the lack of Mo, Mn and Fe had a small effect on nitrate accumulation (Crawford, 1961).

Work on the effect of pH on nitrate accumulation has been lacking. In Smith's work (1968), it was shown that pH has little effect on nitrate accumulation. There was just a small increase in nitrate accumulation with an increase in pH.

c. Soil Moisture

Serious nitrate poisoning has been reported from drought - prevailing areas. Several moisture-dependent processes evidently contribute to the accumulation of nitrate. To a large extent this accumulation is believed to be attributed to the disturbances in nitrate assimilation in the plant. The rate of nitrate reduction is slowed due to a stress in nitrate reductase activity because of a lack of moisture. However, a long continuous drought is not likely to increase nitrate accumulation (Gilbert, 1946).

Experiments on the effect of water stress on nitrate accumulation are lacking. In corn the nitrate content of plants under drought condition was found to be almost double than in those grown under irrigation (Flynn, 1957). Similar effects have been shown by Hanway and Englehorn (1958) in corn and soybean and by Gonske and Keeney (1969) working with corn.

d. Other Factors

Several other factors, such as light, temperature and herbicide, are known to affect nitrate accumulation. However, the mechanism by which these factors exert their influence are not well understood.

Light has long been implicated as a factor in nitrate metabolism. The influence of light intensity and quality has been investigated. In several experiments it was shown that a reduction in light intensity is associated with nitrate accumulation. As an example, in white clover 4,632, 6,920, and 9,270 ppm of nitrate - N accumulated when plants were grown under 100, 50 and 20% of light, respectively (Bathrust and Mitchell, 1958). In corn the nitrate - N content was twice as much in plants grown under 35% shade as in those grown unshaded (Knipmeyer, 1962). Duration of illumination was shown to influence nitrate content in oats; plants given continuous light contained only one-third as much as those receiving only daylight (Whitehead, 1948). A clear-cut mechanism by which temperature influences nitrate accumulation is not understood. Nightingale (1930) showed that two comparable soybean plants grown at different temperatures differed in nitrate content. The nitrate content was higher in plants grown at the lower temperature. On the other hand, Bathrust (1958), reported a different trend; he found that at the higher temperature, nitrate accumulation was higher. He did not offer any explanation, but Nightingale attributed it to the reduction of nitrate assimilation due to low temperature. In another experiment, George et al. (1971) showed that both effects were possible. The nitrate content was increased with an increase in temperature if the plants were subjected to a short treatment period (less than one week); however, when the treatment was extended to more than a week, the highest nitrate content occurred in plants grown under the lowest temperature. It was suggested that under the long term treatment the high temperature tended to produce an increase in growth and perhaps produce a dilution effect (George, 1971).

A satisfactory generalization regarding the influence of herbicide on nitrate accumulation is not available. However, several reports have been encountered indicating that such an effect does exist. In 1950 Stahler and Whitehead (1950) reported that sugar beet leaves sprayed with 2, 4 - D contained 20 times as much nitrate as leaves of untreated plants. Doll and Meggitt (1968) found that at low temperature atrazine induced accumulation of nitrate in corn. In a more recent work by Allinson and Peters (1970), it was shown that the nitrate content of canarygrass was higher in plots treated with simazine than in the control plot. It was also concluded that an increase in nitrate was evident, especially when a high N level was combined with a high simazine level (Allinson and Peters, 1970).

MATERIALS AND METHODS

The study consisted of two parts: a survey of the nitrate content of plant species from different locations, and a greenhouse experiment to study the effect of inorganic N fertilizer and cattle manure on nitrate accumulation.

A. Survey of Nitrate Accumulators

Plant samples were collected from two locations; the University of Hawaii Manoa Campus and the Foremost Dairy Farm, Waimanalo, Oahu. The former represents a grass and shrub area that did not receive any fertilizer application, while the latter represents different cultivated fields which had received different levels of manure. At the latter location, plant samples were collected from Fields 6 and 7 (FD-6 and FD-7), the "Mauka" field (FD-M) and the "Virgin" field (FD-V). FD-6 and FD-7 plots received the highest amounts of manure among the three fields. In these two fields the manure was applied as a slurry at six weeks intervals. FD-M plot received dry manure at the time of planting the forage, while FD-V was uncultivated and did not receive any manure application.

Plant samples from the two locations were randomly harvested at about 2 inches above the soil surface. They were identified taxonomically and descriptions, such as location, stage of maturity and the overall condition of the plants were recorded. The samples were dried in a blower oven at 65°C. The dried samples were then ground in a Wiley Mill and passed through a 40-mesh sieve. Finally, the samples were analyzed for their nitrate content by the nitrate electrode method as described in a later section.

B. Greenhouse Experiments

In the greenhouse study, two sets of experiments were carried out. These were experiments on the effects of (1) inorganic N fertilizer and (2) cattle manure on nitrate accumulation in spinach (*Spinacia oleracea* (L.)), spiny amaranth (*Amaranthus spinosus* (L.)) and sudangrass (*Sorghum bicolor* var. sudanense (L.) Moench).

1. Description of Soils Used in the Experiment

Two surface soils were used in the greenhouse trials. One was the Wahiawa silty clay (Tropeptic Eutrustox) and the other was the Waialua silty clay (Vertic Hapustoll). The Wahiawa soil was collected from the University of Hawaii Experimental Farm at Poamoho, Oahu. This soil is derived from basalt and are predominantly kaolinitic in mineralogy. The Waialua soil was collected from the University of Hawaii Experimental Farm at Waimanalo, Oahu. This soil is derived from alluvium and the important secondary minerals are 2:1 expanding clay and kaolinite. The soils differ in their chemical, physical and mineralogical properties. Some of the properties of these soils are given in Table 1.

Table 1. Some properties of soils used in the greenhouse experiment

Soil Type	pH	Total-N %	NO_3-N ppm	NH_4-N ppm
Wahiawa silty clay	5.2	0.18	5.26	24.52
Waialua silty clay	6.3	0.22	41.17	14.97

2. Experiment 1. Effects of Inorganic N Fertilizer on Nitrate Accumulation

The Wahiawa and the Waialua soils were used in the experiment on the effects of inorganic N fertilizer on nitrate accumulation. The N rates used were 0, 500 and 2,000 lb/A (0, 560 and 2,240 kg/ha). The N was applied as $Ca(NO_3)_2 \cdot 4H_2O$ in split applications; one-half of the N was applied before planting, and the other half was applied in three split applications at 10 day intervals. The first split application was made at three weeks after planting. Prior to planting, other nutrients were also applied; their rates and carriers are shown in Table 2. All nutrients were applied in solution form except $Ca(OH)_2$ which was applied in the solid powder form.

Table 2. The rates and carriers of other (than N) nutrients applied prior to planting

Nutrient	Amount Applied (lb/A)		Carrier
	Wahiawa	Waialua	
P	1200	600	$NaH_2PO_4 \cdot H_2O$
K	800	800	KH_2PO_4
Mg	100	100	$MgSO_4 \cdot 7H_2O$
Fe	100	100	$FeSO_4 \cdot 7H_2O$
Mo	1	1	$Na_2MoO_4 \cdot 2H_2O$
B	10	10	$Na_2B_4O_7 \cdot 10H_2O$
Cu	5	5	$CuSO_4 \cdot 5H_2O$
Zn	20	20	$ZnSO_4 \cdot H_2O$
$Ca(OH)_2$	500	-	$Ca(OH)_2$

This experiment included three species of plants: spinach, sudangrass and spiny amaranth. The plants were seeded in 2 1/2 gallon plastic pots. At the end of two weeks the spinach and spiny amaranth were thinned to four uniform seedlings, while the sudangrass was thinned to ten. The pots were arranged in a split-plot design with the three plant species as the main plots. The treatments were replicated three times.

At the end of 60 days, the plants were harvested. They were cut at about an inch from the soil surface, weighed, and dried in a blower oven at 65°C. The dried samples were weighed, ground in a Wiley Mill and analyzed for nitrate - N and total - N.

A ratoon crop of sudangrass was allowed to grow for further testing. Additional amounts of N were added to maintain the original levels of N at 0, 500, and 2,000 lb/A. The amounts of N added were equal to the amounts removed by the first crop. Ratoon plants were harvested at the end of 42 days, dried, ground and analyzed for nitrate - N and total - N.

3. Experiment 2. Effects of Cattle Manure on Nitrate Accumulation

The test included two soils, the Wahiawa and the Waialua, and three species of plants, spinach, sudangrass and spiny amaranth. The test was conducted in a similar manner as in Experiment 1 except that cattle manure was used as the N carrier. The various N contents of the manure are given in Table 3.

Table 3. N content of the cattle manure used in the experiment.

Material	Total-N %	NO_3-N (ppm)	NH_4-N (ppm)	Moisture (%)
Cattle Manure	4.28	31.13	2093	57

Cattle manure at four rates viz. 0, 50, 100 and 200 T/A[1/] (0, 56, 112 and 224 t/ha) were tested. The manure was thoroughly mixed with the soil at two weeks prior to planting. Other nutrients were also applied at the same rates as in Experiment 1. The plants were seeded in 5-gal. plastic pots and thinned to six plants after two weeks, except the sudangrass which was thinned to ten. The treatments were replicated three times and the pots were arranged in a randomized complete block design. Split-plot design was not used because from the earlier experiment it was found that different plant species were uniform in size.

The plants were harvested after 50 days, dried in a blower oven at 65°C, weighed, ground and analyzed for NO_3-N and total-N.

C. Analytical Procedures

1. Analysis of Nitrate-N in Plant Samples

The analysis of nitrate - N was carried out by using a nitrate specific ion electrode as described by Paul and Carlson (1968).

A 0.5 g sample of finely ground plant material was placed in a 125 ml Erlenmeyer flask; 50 ml of distilled water, 1 ml of Al resin and 1 ml of Ag resin were added into the flask. The flask was stoppered and shaken for 1 hr. The suspension was filtered through a Whatman #2 folded filter paper into a 100 ml breaker. The mv reading was then taken by using an expanded pH meter (Beckman Expandomatic SS2) with a Nitrate Ion Electrode Model 92 - 07 (Orion Research).

[1/]Originally, only three rates were to be tested, viz. 0, 50 and 200 T/A. However, after two weeks the sudangrass with the 200 T/A treatment showed a severe retardation in growth and abnormal leaves. These adverse effects were suspected to be due to salt injury. For fear that these plants would die, an additional rate of 100 T/A was introduced. A new Wahiawa soil sample was collected from the field since the earlier sample was insufficient.

The Ag resin was added to remove the Cl^- ion which would interfere in the analysis, while the Al resin was added to reduce the bicarbonate and organic anion interference. The resin was prepared by adding about 70 g of $AgNO_3$ to 100 g of Dowex 50-x8 resin, (50 - 100 mesh in hydrogen form) in a beaker. Distilled water was added and the mixture was carefully mixed. It was then filtered under low pressure and rinsed with distilled water. Presence of $AgNO_3$ in the filtrate was checked by using NaCl solution. Rinsing was continued until no AgCl precipitate was evident. A similar method was employed for preparing Al - resin, except that $Al_2(SO_4)_3$ was used instead of $AgNO_3$ and the filtrate was tested with BaCl for traces of SO_4^{-2}.

2. Total - N in Plant Sample

The total - N in plant samples was determined by the method described by Peterson and Chester (1964).

A 0.5 g of finely-ground plant sample was placed in an 800 ml Kjeldahl flask and 10 ml of distilled water, 10 ml of 5% (w/v) $KMnO_4$ and 20 ml of 50% (v/v) H_2SO_4 were added in the indicated sequence. The sulfuric acid was added slowly with shaking. After 5 minutes, 5 g of powdered reduced iron was added. The flask was capped with a beaker and allowed to stand until the effervesence ceased. The sample was heated gently at low heat for 1 hr. The sample was then cooled. A digestion mix (10 g K_2SO_4, 1 g $CuSO_4$ and 1 mg Se) and 30 ml of concentrated H_2SO_4 were added to the sample and the mixture was digested for 1 1/2 to 2 hrs. following the appearance of a greenish yellow color. The digested sample was diluted to a volume of 500 ml in a volumetric flask. Then an aliquot of 10 ml was pipetted and placed in a distillation flask, and 10 ml of 10N NaOH was added. The distillation flask was immediately connected to the distillation apparatus and the distillate was collected in a 4% boric solution. When the distillation was completed, the distillate was titrated with standard sulfuric acid to determine the total - N content.

3. Measurement of Electrical Conductivity of Soil

The Ec reading of the soil was made in a soil-water extract at a 1:1 ratio. A 25 g of soil sample was placed in a 250 ml Erlenmeyer flask, 25 ml of distilled water was added, the flask was stoppered, and agitated on a mechanical shaker for 15 minutes. It was then allowed to stand for an hour and then was filtered. The Ec reading of the extract was obtained using a Wheatstone Bridge.

4. Statistical Analysis

The experimental data were analyzed statistically by the method of "analysis of variance" as given by Snedecor and Chocran (1972). The means were compared using the Duncan's multiple range test at a 0.05 level of significance (Duncan, 1955).

RESULTS AND DISCUSSION

A. Survey of Nitrate Accumulators

1. Nitrate Content of Some Common Weeds and Forage Crops

Many weeds found in cultivated fields and pasture lands may contain a considerable amount of nitrate. Such weeds are a potential hazard because of their possible inclusion in the harvested forage or their being eaten by grazing animals in pastures. In this study some common weeds from different locations were collected and analyzed for their nitrate content.

The nitrate content of some common weeds collected from the University of Hawaii Manoa Campus is given in Table 4. The nitrate - N content ranged from 200 to 4150 ppm. In Wyoming, Gilbert (1946) compiled the analysis of nitrate content of a wide range of weed species and reported a range from traces to 8.3% KNO_3 (1.15% NO_3-N).

Among the species analyzed, spiny amaranth (Amaranth spinosus (L.))[1/] was the highest nitrate accumulator, having a nitrate - N content of 4150 ppm. This is in accord with Gilbert (1946) who reported that red root pigweed (Amaranth spp.) belonging to the same genus as spiny amaranth was among the highest nitrate accumulators. It was also reported by other earlier workers (Woo, 1919; Wilson, 1943; Olson and Eugene, 1940; Sund, 1957) that the same plant (red root pigweed) contained a high concentration of nitrate. Mention was also made previously of animal losses resulting from animals consuming hay containing this plant (Bradley, 1940). Other weeds, such as bristly foxtail

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[1/] The scientific name of the plant will be given only when it appears for the first time.

Table 4. The NO_3-N content of some common weeds collected at University of Hawaii Manoa Campus

Species	NO_3-N
	ppm
Scarlet-fruited passion flower (Passiflora feotida (L.) Var.)	940
Spiny amaranth (Amaranthus spinosus (L.))	4150
Flora paintbrush (Emilia sonchifolia (L.) DC.)	300
Wiregrass (Eluesine indica (L.) Gaertn.)	740
Bristly foxtail (Seteria verticillata (L.) Beauv.)	2900
Radiate fingergrass (Chloris radiata (L.) Swartz.)	600
Sensitive plant (Mimosa pudica (L.))	310
Sandbur (Cenchrus echinatus (L.))	330
Spanish needle (Biden pilosa (L.))	220
Pigweed (Protulaca oleracea (L.))	3000
Graceful spurge (Euphorbia glomerifera (Millsp.) L. C. Wheeler)	260
Honohono (Commelina diffussa Burm. f.)	840
Guineagrass (Panicum maximum Jacq.)	2600
Natal redtop (Rhynchelytrum repens (Willd.) C. E. Hubb.)	340
Five-fingered morning glory (Impomoa cairica (L.) Sweet)	500
Redtop (Agrotis alba (L.))	330

(*Seteria verticillata* (L.) Beauv.), pigweed (*Portulaca oleracea* (L.)) and guineagrass (*Panicum maximum* Jacq.), appear from this study (Table 4), to be potentially toxic. The rest of the weeds seem to be low nitrate accumulators and would most probably not pose any danger of nitrate poisoning under normal conditions.

The second group of plant samples were collected from the Foremost Dairy and near its immediate vicinity in Waimanalo, Oahu. These samples were collected from different forage plots that have different levels of dairy manure applied. The content of nitrate - N in some common weeds collected from an uncultivated field (FD - V) is given in Table 5. This plot did not receive any manure application. The NO_3-N content of the samples collected from this plot ranged from 460 to 4,900 ppm. Spiny amaranth was the most common weed in this location and as in the earlier collection at the Manoa Campus, it was also the highest nitrate accumulator with a nitrate - N content of 4,900 ppm. Other species that contained over 1,000 ppm nitrate - N were pigweed (*Portulaca oleracea* (L.)), paragrass (*Bracharia mutica* (Farsk) Stapf) and wiregrass (*Eluesine indica* (L.) Gaertn.). However, in this Waimanalo location, bristly foxtail (*Seteria verticillata* (L.) Beauv.) had a nitrate - N concentration of 720 ppm compared to 2,900 ppm in the sample collected from the University of Hawaii Manoa Campus. The other species that were collected from the two areas seemed to have comparable values. Besides spiny amaranth, pigweed (*Portulaca oleracea* (L.)) was consistently high in nitrate content at both locations. However, this species of weed was not abundant in either location.

The nitrate content of some weeds and forage crops collected from Plots FD6 and FD7 is given in Table 6. These fields had received

Table 5. The NO_3-N content of some common weeds collected from an uncultivated field (FD-V) at Foremost Dairy Farm Waimanalo, Oahu

Species	NO_3-N
	ppm
Spiny amaranth (Amaranthus spinosus (L.))	4900
Ageratum (Ageratum conyzoides (L.))	620
Wiregrass (Eluesine indica (L.) Gaertn.)	1150
Paragrass (Bracharia mutica (Forsk.) Stapf.)	2700
Japanese tea (Cassia mimosoides (L.))	500
Honohono (Commelina diffusa Burm. f.)	600
Bermudagrass (Cynodon dactylon (L.) Pers.)	460
Pigweed (Protulaca oleracea (L.))	4000
Bristly foxtail (Seteria verticillata (L.) Beauv.)	720

FD-V: A "virgin" field that was not cultivated and did not receive any manure application.

a heavy application of dairy manure slurry once every six weeks since 1970. No commercial fertilizer had been applied. The fields were planted with forage crops which are used to feed the animals on the farm. The FD-7 was planted with sudax (Sorghum bicolor (L.) Moench. X Sorghum bicolor var. sudahense (L.) Moench.), while the FD-6 plot was planted with paragrass (Bracharia mutica (Forsk.) Stapf.). At the time when the plant samples were collected, both grasses were growing very well. The fields were generally heavily covered with weeds, especially the sudax plot (FD-7). The most common weed found in this area was spiny amaranth.

The nitrate - N content of the forage crops and the weeds in these two fields were very high (see Table 6). The two forage crops, sudax and paragrass had nitrate - N levels of 8,000 ppm and 4,000 ppm, respectively. Many workers (Emerick, 1963; Gillingham, 1969; Ryan, 1972) consider a nitrate - N content of 1,500 ppm (O.D basis) or above in forage to be toxic for livestock consumption. As evident from the analysis, these two forage crops have a nitrate - N content very much higher than the "safe" level. Thus this forage if fed to animals may cause acute nitrate poisoning.

The highest nitrate accumulator in these plots (FD-6 and FD-7) was the spiny amaranth as in the previous collections. Under this condition of receiving periodic manure slurry application, the nitrate - N content was 11,500 ppm, which is three times higher that in samples collected from the untreated plot (FD-V). As stated earlier, it was the most common weed found in this area and thus the chance of its inclusion in the forage was great.

Table 6. The NO_3-N content of some common weeds and forage crops from Fields FD-6 and FD-7 at Foremost Dairy Farm Waimanalo, Oahu

Species	NO_3-N
	ppm
Sudax (Sorghum bicolor (L.) Moench. X Sorghum bicolor var. Sudanense (L.) Moench.)	8000
Spiny amaranth (Amaranthus spinosus (L.))	11500
Paragrass (Bracharia mutica (Forsk.) Stapf.)	4000
Ageratum (Ageratum conyzoides (L.))	3900
Wiregrass (Eluesine indica (L.) Gaertn.)	4100
Bristly foxtail (Seteria verticillata (L.) Beauv.)	6000
Bermudagrass (Cynodon dactylon (L.) Pers.)	1100
Japanese tea (Cassia mimosoides (L.))	2100
Pigweed (Portulaca oleracea (L.))	7000

FD-6 and FD-7: These fields received heavy applications of manure slury; once in every six weeks.

The nitrate content of the other weed species in these fields was above 1,000 ppm (Table 6). Pigweed and bristly foxtail, which were shown to be high accumulators in the previous collection, were also among the high accumulators in this location. Many species of weeds, such as ageratum (Ageratum conyzoides (L.)), Japanese tea (Cassia mimosoides (L.)), bermudagrass (Cynodon dactylon (L.) Pers.) and wiregrass (Eluesine indica (L.) Gaertn.), which do not accumulate nitrate under normal conditions were found to have nitrate - N levels doubled or tripled when grown in fields receiving manure. Therefore, the inclusion of these weeds in animal feed can create a nitrate - poisoning problem.

Plant samples were also collected from the "Mauka" Field (FD-M). This field, which was planted with guineagrass (Panicum maximum Jacq.), received an application of cattle manure only at the time of planting. Similarly, commercial fertilizer was not used in this field. However, this field did not receive as much manure as Fields FD-6 and FD-7. The nitrate content of the forage crop and weeds growing in this field is given in Table 7. The nitrate - N content in the samples from this "Mauka" Field is higher than in the samples from the untreated "virgin" field (FD-V), but lower than in samples collected from the fields receiving heavy applications of manure slurry (FD-6 and FD-7). The forage crop, guineagrass, growing in this field have a nitrate - N content of 1,500 ppm which makes this forage unsuitable for animal consumption. The spiny amaranth was still a high nitrate accumulator, with a nitrate - N content of 4,000 ppm. As in the other fields, this species was the most common weed

Table 7. The NO_3-N content of some common weeds and forage crops collected from the "Mauka" Field (FD-M) at Foremost Dairy Waimanalo, Oahu

Species	NO_3-N
	ppm
Spiny amaranth (Amaranthus spinosus (L.))	4000
Bristly foxtail (Seteria verticillata (L.) Beauv.)	1900
Wiregrass (Eluesine indica (L.) Gaertn.)	1900
Ageratum (Ageratum conyzoides (L.))	450
Guineagrass (Panicum maximum Jacq.)	1500
Scarlet-fruited passion flower (Passiflora feotida (L.) Var.)	2200
Palmgrass (Seteria palmifolia (Koen.) Stapf.)	1700
Paragrass (Bracharia mutica (Forsk.) Stapf.)	5000
Bermudagrass (Cynodon dactylon (L.) Pers.)	600

FD-M: This field received an application of manure at the time of planting.

occurring in this location. Other species of weeds from this "Mauka" Field, such as bristly foxtail (Seteria verticillata (L.) Beauv.), wiregrass (Eluesine indica (L.) Goertn.), scarlet-fruited passion flower (Passiflora feotida (L.) var.) and paragrass (Bracharia mutica (Forsk.) Stopf.), were found to have a high concentration of nitrate - N and can be potentially toxic.

The practice of applying animal manure to forage crop has been carried out on this farm for several years. This practice may be considered desirable from the standpoint of management because it eliminates the problem of waste disposal and also helps to reduce cost of forage production since commercial fertilizer is not used at all. However, as evident from the analyses, the nitrate - N content in plants is greatly increased by manure application. This trend was also shown by Hanway and Englehorn (1958) in Iowa. This is not desirable because of its hazard to animals. Cattle manure should be applied with caution and excessive applications should be avoided.

2. Variation of Nitrate - N Content with Stage of Maturity and Part of Plant

Two species of plants, viz. sudax and spiny amaranth, were collected at different stages of maturity and analyzed for nitrate - N content. In addition, the spiny amaranth was separated into leaves and stems and the nitrate - N content was analyzed separately. The variation of nitrate content with stage of maturity and part of plant is given in Table 8. Sudax had a higher nitrate - N content at the earlier then the later stage of maturity. This is in agreement with several earlier reports on forage crops (Lovelace, 1968; Crawford,

Table 8. The NO_3-N content of sudax and spiny amaranth at different stages of maturity and of different parts of plant

Species	NO_3-N Young	NO_3-N Old
	ppm	
Sudax (composite)	8,000	700
Spiny amaranth (composite)	6,000	11,500
Spiny amaranth (leaf)	2,200	1,400
Spiny amaranth (stem)	20,000	26,000

1961; George, 1971). However, the spiny amaranth showed an opposite trend; the nitrate - N content was higher in the older plants than in the younger plants, with the exception of the nitrate - N content of the leaves. Although it is known that nitrate - N is higher in the younger than in older plants, it has been stated by Wright and Davidson (1964) that the nitrate - N content in plants first rises, and then after reaching a peak at a certain stage, declines as plants mature. This latter statement may help explain the trend in spiny amaranth. Perhaps the younger plants were too young to accumulate sufficient nitrate, while the older plants were at the stage where nitrate accumulation was at its peak. The nitrate - N content also varied with different parts of the plant.

The stem contained a much higher nitrate content than the leaf. This observation was also reported in corn, sorghum and oats (Whitehead, 1948; Flynn, 1957; Hanway and Englehorn, 1958).

B. Effects of Inorganic N Fertilization on Nitrate Accumulation

This investigation attempts to provide information on the effects of N fertilization on nitrate accumulation in three tropical species, viz. spinach (*Spinacia oleracea* (L.)), sudangrass (*Sorghum bicolor* var. sudanense (L.) Moench.) and spiny amaranth (*Amaranthus spinosus* (L.)). These species were reported by earlier workers (Gilbert, 1946; Barker, 1973; Murphy, 1967) as being high nitrate accumulators; however, these reports were based on temperate conditions. Such work in the tropics is lacking.

1. Nitrate Content

The nitrate - N contents of sudangrass, spinach and spiny amaranth as affected by varying rates of N fertilization are presented in Tables 9 and 10. Nitrate accumulation was significantly affected by plant species (Table 11). This tendency for nitrate - N to vary with different plant species in this study agrees with the results reported in several earlier papers (Murphy and Smith, 1967; Crawford, 1961; Gilbert, 1946; Hanway and Englehorn, 1958). Of the three species reported, spinach accumulated the highest amount of nitrate - N. Spinach and spiny amaranth are somewhat similar in their trends to accumulate nitrate, while sudangrass tended to accumulate less nitrate than the other two species. Without the application of N, however, these three species had similar nitrate - N levels. The difference in nitrate - N content among the three species was more obvious when N was applied and the largest difference occurred at the highest level of N applied.

The accumulation of nitrate - N was directly related to N fertilization in all three species. The effect of N application on the nitrate content of the three species studied was highly significant (Table 11); however, the increments of increase among the species differed in magnitude. In sudangrass the increment of nitrate - N was not significant when 500 lb N/A was applied in comparison with the other species with exception of spiny amaranth grown in the Wahiawa soil. The nitrate - N values of sudangrass at this 500 lb level of N are lower than the values reported by Murphy and Smith (1967). These values are also lower than those reported by Sumner *et al.* (1965). Both of these latter reports, however, were based on field trials. Moreover, different forms of N fertilizer were used; other nutrients

Table 9. The NO_3-N and total-N content of sudangrass, spinach and spiny amaranth grown in Wahiawa soil at different levels of N fertilization

Species	N applied	NO_3-N	Total-N	NO_3-N/Total-N
	lb/A	%		
Sudangrass	0	0.060^a	0.32^a	18.75
	500	0.068^a	0.73^a	9.32
	2000	0.513^b	1.97^b	26.04
$\bar{x}$	-	0.214	1.01	-
Spinach	0	0.059^a	1.14^a	5.17
	500	0.103^b	1.67^a	6.17
	2000	1.370^c	3.33^b	41.14
$\bar{x}$	-	0.511	2.05	-
Spiny amaranth	0	0.051^a	1.03^a	4.95
	500	0.079^a	1.40^a	5.64
	2000	1.167^b	3.46^b	33.73
$\bar{x}$	-	0.432	1.96^b	-

Means for a single species followed by different letters are significantly different at the 0.05 level.

Table 10. The NO_3-N and total-N content of sudangrass, spinach and spiny amaranth grown in Waialua soil at different levels of N fertilization

Species	N applied		Total-N	NO_3-N/Total-N
	lb/A	%		
Sudangrass	0	0.059[a]	0.33[a]	17.87
	500	0.084[a]	0.75[a]	11.20
	2000	0.707[b]	2.12[b]	33.35
$\bar{x}$	-	0.283	1.07	-
Spinach	0	0.053[a]	1.19[a]	4.45
	500	0.167[b]	1.84[b]	9.08
	2000	2.413[c]	4.25[c]	56.78
$\bar{x}$	-	0.878	2.43	-
Spiny amaranth	0	0.049[a]	1.27[a]	3.83
	500	0.167[b]	2.12[b]	7.88
	2000	1.667[c]	4.08[c]	40.86
$\bar{x}$	-	0.628	2.49	-

Means for a single species followed by different letters are significantly different at the 0.05 level.

Table 11. Analysis of variance of the NO_3-N concentration of three plant species grown in two soils with three rates of N fertilization

Source of variation	DF	Mean square
Main plot		
Blocks(r)	2	-
Species(Sp.)	2	0.9435935**
Error a	4	0.0319417
Sub-plot		
Soil(S)	1	0.611629**
Nitrogen(N)	2	8.952970**
S X N	2	0.470029*
S X Sp.	2	0.0929458
Sp X N	4	0.8294582**
S X Sp X N	4	0.0857307
Error b	30	0.0697909

*Significance at the 0.05 level of probability

**Significance at the 0.01 level of probability

were not applied; and the plant age at which the analysis was made was not reported. The comparatively lower content of nitrate accumulated in sudangrass perhaps can be attributed to the high application of other nutrients (see Table 2) and the late stage of maturity (60 days) when the analysis was made.

The nitrate - N content of sudangrass was increased almost ten times when N application was increased from 500 to 2,000 lb N/A in both soils (Tables 9 and 10). The highest nitrate - N was 0.707% in plants grown in the Waialua soil at the 2,000 lb N/A rate. If a nitrate - N content of 0.15% (O.D. basis) is considered as the toxic level of forage use for livestock feed (Ryan, 1972; Bradley, 1940; Emerick, 1962), then an application of 500 lb - N/A would not pose any nitrate-poisoning problem. However, earlier reports (Sumner, 1965; Murphy and Smith, 1967) showed that the accumulation of nitrate - N in toxic amounts in sudangrass occurred when N application was much lower than 500 lb N/A.

In spinach the nitrate - N content in the plant tissue was considerably increased with an addition of 500 lb N/A (see Tables 9 and 10). The magnitude of the nitrate - N increase differed between plants grown in the two soils. The nitrate - N content was increased to about three times in spinach grown in the Waialua soil, while the nitrate - N content of plants grown in the Wahiawa soil was increased only by about two times (Tables 9 and 10 and Fig. 1 and 2). Barker et al. (1971) studied the effects of N fertilization on two varieties of spinach and reported that an addition of 450 kg N/ha (400 lb N/A) produced an increase of nitrate - N content in the plant tissue that was also about double that of the control. Their trend seemed to agree very well with the trend observed in this study; however, their data showed that the nitrate - N contents of the tissue were higher than the values obtained in this experiment.

A further addition of up to 2,000 lb N/A increased the plant tissue nitrate - N content of spinach by more than 10 times (see Tables 9 and 10). The increase in the nitrate - N content is again greater in plants grown in the Waialua soil than in the Wahiawa soil. The highest nitrate - N was found to be 2.43% in plants grown in the Waialua soil with 2,000 lb N/A applied.

It has been estimated that processed spinach for baby food should not contain more than 300 ppm nitrate, which is equivalent to 67 ppm nitrate - N, (Simon, 1966). This tolerance level is apparently very low, for only nitrate levels of spinach from the control plots in this study approached this standard. With N applications, the levels of nitrate - N of spinach in this study were very much higher than the tolerance level. Other estimates, however, indicate that the tolerance level may be considerably higher. It was estimated (Brown and Smith, 1966) that an adult weighing 70 kg would need to ingest 0.7 to 1.0 g of nitrate - N to attain a toxic level. This amount would be contained in 700 to 1,000 g of fresh spinach with a nitrate - N content of 0.1% on the fresh weight basis. An infant needs much less spinach for a toxic dose, possibly as little as 50 g of fresh spinach. Cooking of spinach extracts 75 to 80% of the nitrate (Phillips, 1968).

The nitrate - N content of the spiny amaranth grown in the Waialua soil was increased by almost three times with an application of 500 lb N/A as compared to the control (Table 10). This treble increase was also earlier found with spinach. This nitrate - N content, however, was increased to a smaller magnitude in plants grown in the Wahiawa soil (Table 9). The nitrate - N content was further increased about ten times when the rate of N application was increased from 500 to 2,000 lb N/A in both soils. The highest nitrate - N content in the spiny amaranth was found to be 1.67% in plants grown in the Waialua soil at the highest N rate. Due to its great ability to accumulate nitrate and its abundance in pasture lands, these weed species can pose a serious nitrate poisoning problem in livestock.

In all three species the trend by which N applications affected nitrate - N content in the plants seemed to be similar. The slopes of the nitrate - N response curves from 0 to 500 lb N/A and from 500 to 2,000 lb N/A differed (Figures 1-3). In the former case, the response curve has a very small gradient, implying a small increase of nitrate - N content per unit N applied. The gradient rises sharply in the latter case, indicating a higher increase of nitrate - N per unit N applied. This pattern seems to agree with the results in small grains reported by Baker and Tucker (1971).

The nitrate - N levels in each species as influenced by soil type and rate of N fertilization are shown in Figures 1, 2 and 3. The nitrate - N differed significantly ($P \leq 0.01$) between plants grown in the two N - applied soils (Table 11). In all three species the nitrate - N content in the plants grown in the Waialua soil with N applications was higher than the nitrate - N content in plants grown in the Wahiawa soil. This difference occurred perhaps due to differences in the

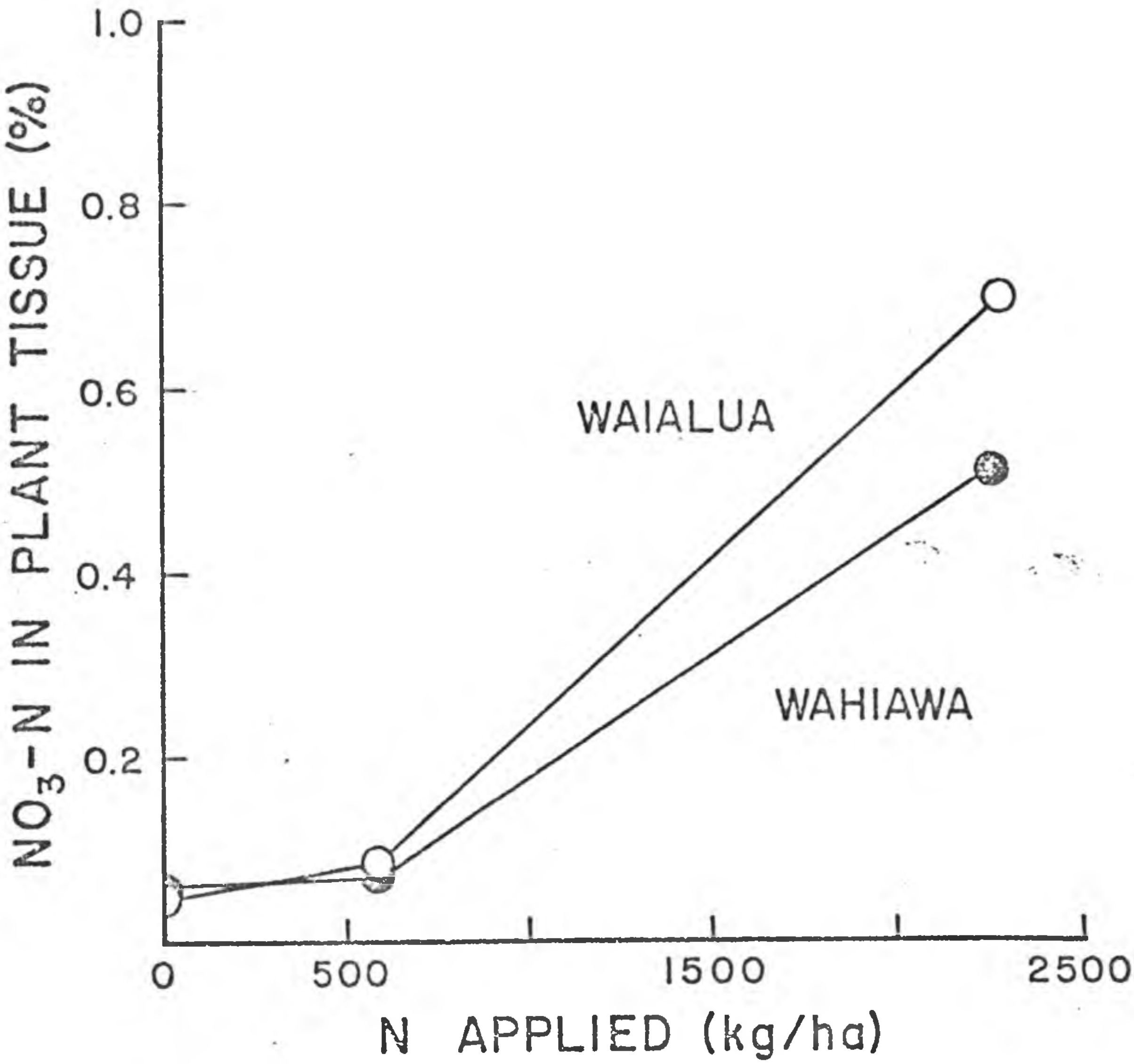

Figure 1. The NO_3-N content of sudangrass grown in the Wahiawa and the Waialua soils at different levels of N fertilization.

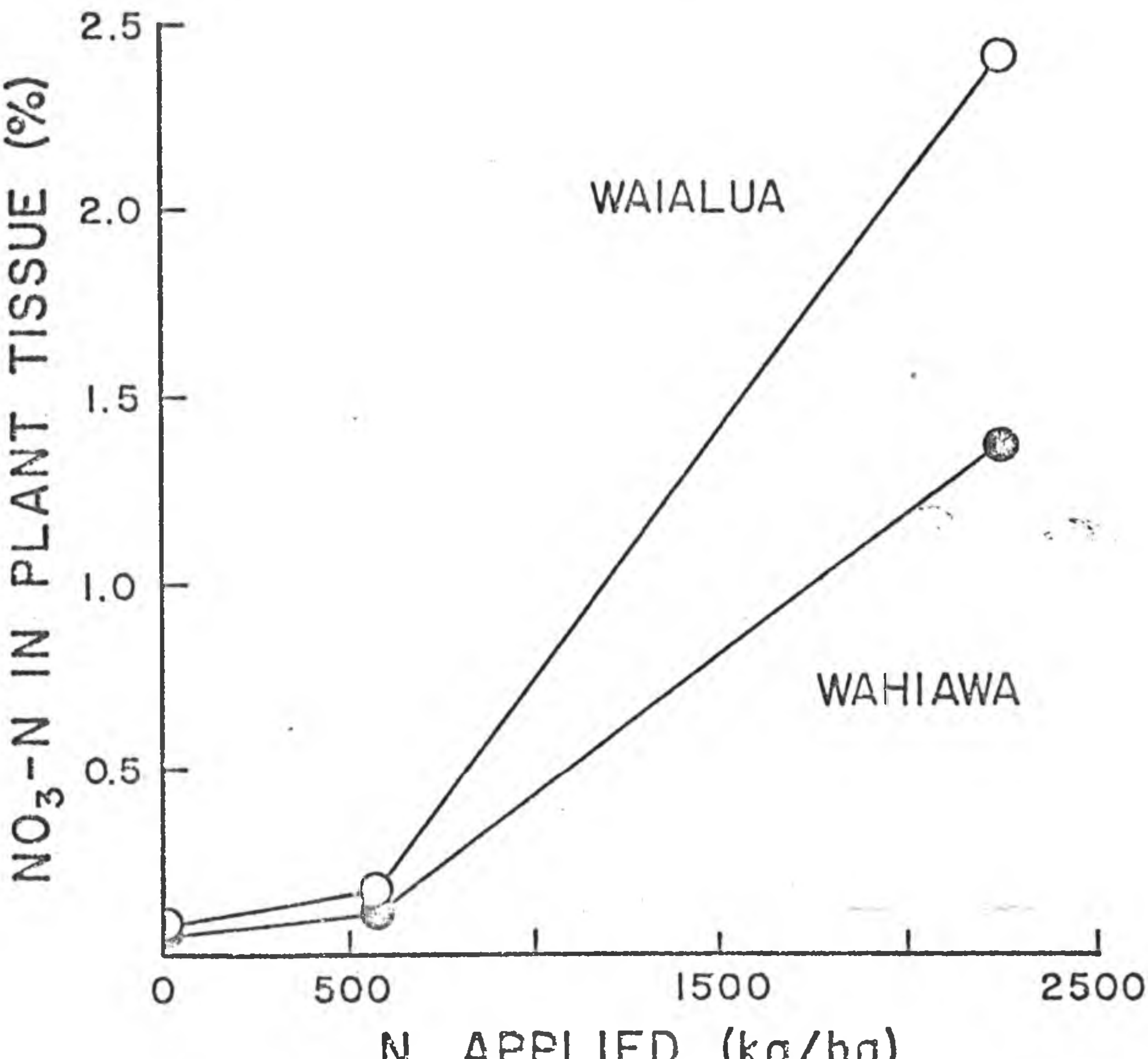

Figure 2. The NO_3-N content of spinach grown in the Wahiawa and the Waialua soils at different levels of N fertilization.

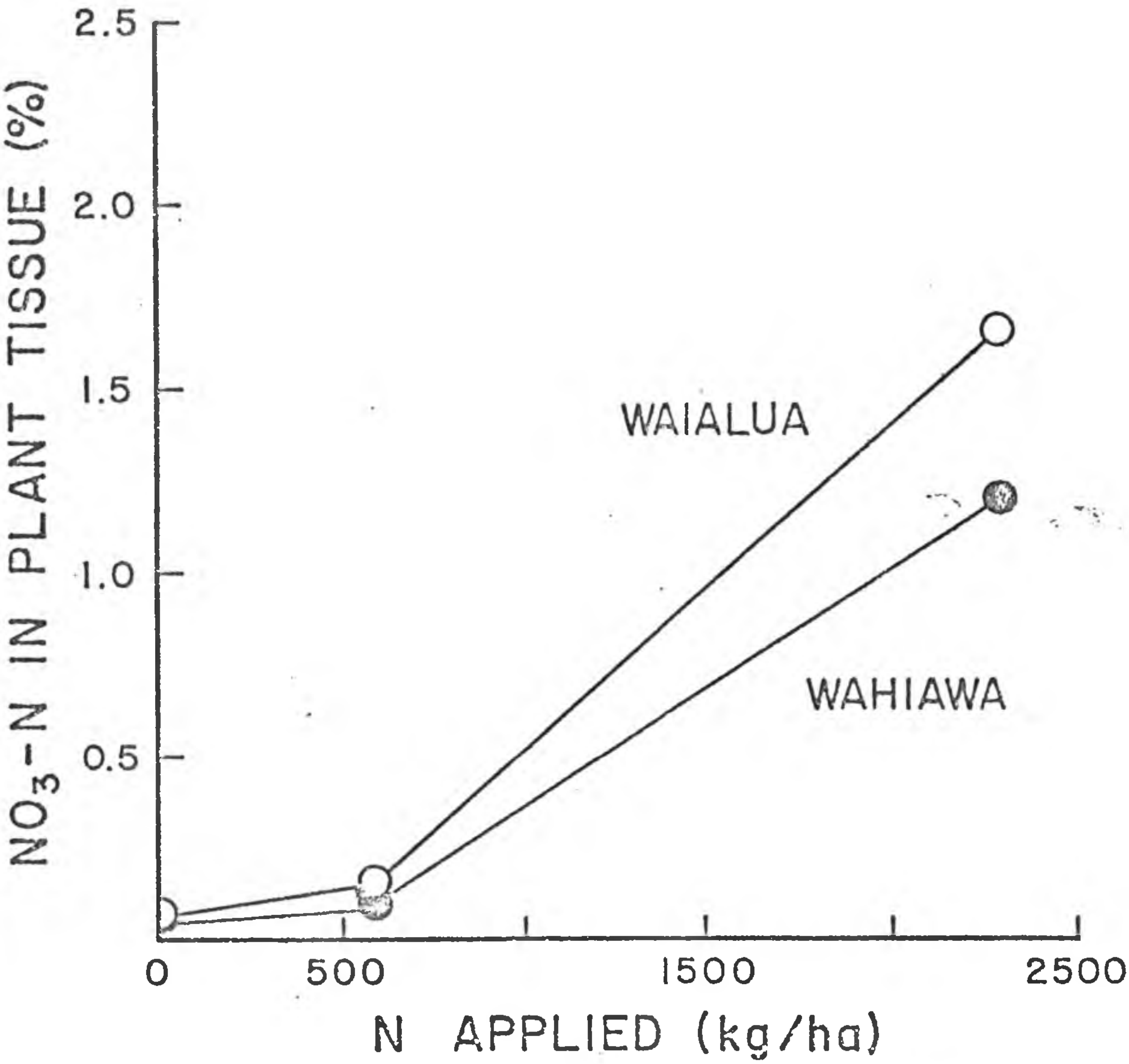

Figure 3. NO_3-N content of spiny amaranth grown in the Wahiawa and the Waialua soils at different levels of N fertilization.

initial soil nitrate - N content, ion exchange capacity, soil texture, or the concentration of other nutrients in the two soils.

The analysis of the soil nitrate - N content (Table 1) indicates that the Waialua soil has a nitrate - N content of 41.17 ppm, which was very much higher than the nitrate - N content of the Wahiawa soil which was 5.26 ppm. The total - N content of these two soils also differ; the Waialua soil has a slightly higher total - N content than the Wahiawa soil (Table 1). These two soils were also shown to have a different rate of N mineralization by Briones (1969). She reported that the Waialua soil has a higher N - mineralization rate than the Wahiawa soil and she also found that N mineralization rate and total - N content of soils were positively correlated.

Although the two soils have high clay contents, the Wahiawa soil behaves more like a slight-textured soil because its clay particles are well-aggregated. Thus, although they both belong to the same textural class, they differ in their physical behavior. The difference in the nitrate - N content of plants grown in these two soils may be attributed to some extent to this "textural" difference. Lovelace (1968) reported that in bermudagrass, soil texture influenced the nitrate - N levels in the plants more than even the rate of N fertilization.

The plants were watered regularly during the growing period. The water was applied in just sufficient amounts to wet the soil so that leaching would not occur; however, in several cases leaching could not be avoided. This resulted in unavoidable differential loss of nitrate - N from the two soils. More nitrate - N would be expected to be leached out from the Wahiawa soil than from the Waialua soil due to the difference in physical characteristic discussed earlier. This difference perhaps can account for the difference in the nitrate - N content of plants grown in the two soils.

2. Total - N

The total - N content of the plant tissue was increased along with the nitrate - N with increasing N application (Tables 9 and 10). However, the total - N trend resulting from N application was different from the nitrate - N trend. The total - N content increased gradually at a constant rate with increasing N applications. This was unlike that of nitrate - N which increased gradually only up to the 500 lb N/A application, while the application of N above 500 lb/A increased plant nitrate content very sharply. It can be inferred from these trends that with an increase of nitrate - N concentration in plant tissue, the rate of nitrate assimilation per unit nitrate absorbed was reduced. This can be clearly understood if the ratio of the nitrate - N to the total - N is examined (Tables 9 and 10). This ratio is obtained by dividing the total nitrate - N by the total - N absorbed by the plants. In all three species, with the exception of sudangrass, the nitrate - N to total - N ratio was increased with an increase in the plant nitrate - N content. In sudangrass the ratio was higher in the control plants than in those plants receiving the 500 lb N/A application. However, with a further increase in N applied, this ratio was increased as in the other two species. The highest ratio recorded was 56.8% in spinach grown in the Waialua soil receiving 2,000 lb N/A. This trend of increasing nitrate - N to total - N ratio with an increase in plant nitrate - N content as demonstrated in this study seems to agree with earlier reports by Smith and Clark (1968) on pearl millet and sudangrass and by Hojjati _et al_. (1972) on corn. However, in most of these reports the amounts of N applied were lower than the rates used in this study.

The total - N content of the plants was significantly affected by the rate of N application, plant species, and soil types (Table 12). These factors were similar to those affecting nitrate accumulation. Of the three species, spinach seemed to accumulate the highest total - N, followed closely by spiny amaranth, while sudangrass had a much lower value (Tables 9 and 10). This trend was also true in the variation of nitrate - N content with plant species discussed earlier.

3. Yield

The vegetative yield of sudangrass, spinach and spiny amaranth as affected by N fertilization and soil types are given in Table 13. The effects of N application, soil type and plant species were significant (Table 14). In all three species, N applications produced an increase in yield. The yield differed significantly between the two soil types. Also, a high nitrate content in plant tissue did not have any detrimental effect on the plant growth.

4. Sudangrass Ratoon Crop

Sudangrass was allowed to grow after the first harvest and the plants were analyzed for nitrate and total - N. The effects of N fertilization on nitrate accumulation in the sudangrass ratoon crop are given in Table 15. As in the original crop, nitrate accumulation was only affected by the levels of N application. The effect of soil types was not significant (Table 16).

Table 12. Analysis of variance of the total-N concentration of three plant species grown in two soils with three rates of N fertilization

Source of variation	DF	Mean Square
Main plot		
Blocks	2	0.537910
Species(S)	2	8.665820**
Error a	4	0.116417
Sub-plot		
Soils(S)	1	1.388810**
Nitrogen(N)	2	26.60629**
S X N	2	0.236895
S X Sp	2	0.248285
Sp X N	4	0.444987*
S X Sp X N	4	0.104150
Error b	30	0.1169973

*Significance at the 0.05 level of probability

**Significance at the 0.01 level of probability

Table 13. Vegetative yield of sudangrass, spinach and spiny amaranth as affected by N fertilization

Species	N applied	Dry matter yield	
		Wahiawa	Waialua
	lb/A	g/pot	
Sudangrass	0	38.3[a]	53.9[a]
	500	91.0[b]	88.3[b]
	2000	116.3[c]	107.7[c]
Sudangrass (ratoon crop)	0	17.7[a]	20.1[a]
	500	28.8[a]	33.9[a]
	2000	65.1[b]	66.7[b]
Spinach	0	5.8[a]	22.2[a]
	500	40.6[b]	75.1[b]
	2000	91.9[c]	108.3[c]
Spiny amaranth	0	7.9[a]	24.7[a]
	500	65.8[b]	79.3[b]
	2000	96.2[c]	97.1[c]

Means for a single species followed by different letters are significantly different at the 0.05 level.

Table 14. Analysis of variance of the dry matter yield of three plant species grown in two soils with three rates of N fertilization

Source of variation	DF	Mean square
Main plot		
Blocks	2	
Species(Sp)	2	3296.120**
Error a	4	65.460
Sub-plot		
Soil(S)	1	1728.36**
Nitrogen(N)	2	27625.59**
S X N	2	240.42
S X Sp	2	482.66**
Sp X N	4	284.64*
S X Sp X N	4	138.93
Error b	30	80.94

*Significance at the 0.05 level of probability

**Significance at the 0.01 level of probability

Table 15. The NO_3-N and total-N contents of sudangrass (ratoon crop) grown at different levels of N fertilization

Soils	N applied	NO_3-N	Total-N	NO_3-N/Total-N
	lb/A	%		
Wahiawa	0	0.074^{a}	1.08^{a}	6.85
	500	0.150^{b}	1.92^{b}	7.92
	2000	0.613^{c}	2.37^{c}	25.86
Waialua	0	0.083^{a}	1.07^{a}	7.76
	500	0.243^{b}	1.86^{b}	13.06
	2000	0.567^{c}	2.40^{c}	23.63

Means for a single species followed by different letters are significantly different at the 0.05 level.

Table 16. Analysis of variance of the nitrate-N concentration of sudangrass ratoon-crop grown in two soils with three rates of N fertilization

Source of variation	DF	Mean square
Blocks	2	
Soils(S)	1	0.002178
Nitrogen(N)	2	0.439194**
S X N	2	0.006518
Error	10	0.00076289

**Significance at the 0.01 level of probability

The increase of nitrate - N with the addition of 500 lb N/A application was more marked in the ratoon crop than in the plant crop. With a further increase of N application from 500 to 2,000 lb N/A, the nitrate - N content was further increased but to comparatively lower values than those obtained in the plant crop.

The effect of soil type was more marked in the plant crop than in the ratoon crop, probably because in the plant crop the effect of N fertilization could have been masked by the initial soil - N (Figures 1 and 4). In the ratoon crop, this effect did not occur because the soil - N was depleted by the original crop. A similar effect of initial soil - N depletion was also observed by Lovelace (1968) with bermudagrass. This probably could also account for the less distinct difference between the effect of soil types. The comparatively lower nitrate content at the 2,000 lb N/A level for the Waialua soil (compare Figures 1 and 4) occurred perhaps because the peak of the nitrate - N content could have occurred below that level. According to Burt (1962) and Crawford (1961) the maximum nitrate - N content in plants occurs at about the 1,000 lb N/A level in soil. Also, in this study the ratoon plants were harvested at about two weeks earlier than the plant crop; this probably could account for the higher nitrate - N content in the ratoon crop (Crawford, 1961; Gonske and Keeney, 1969; Gul and Kolp, 1960).

The vegetative yield of the ratoon crop is given in Table 13. The yield was significantly affected by N application, the effect of soil type on yield was not significant (Table 18). The ratoon yields were much lower than those of the original crop. Thus, the higher nitrate - N concentration in the ratoon crop could have been due to

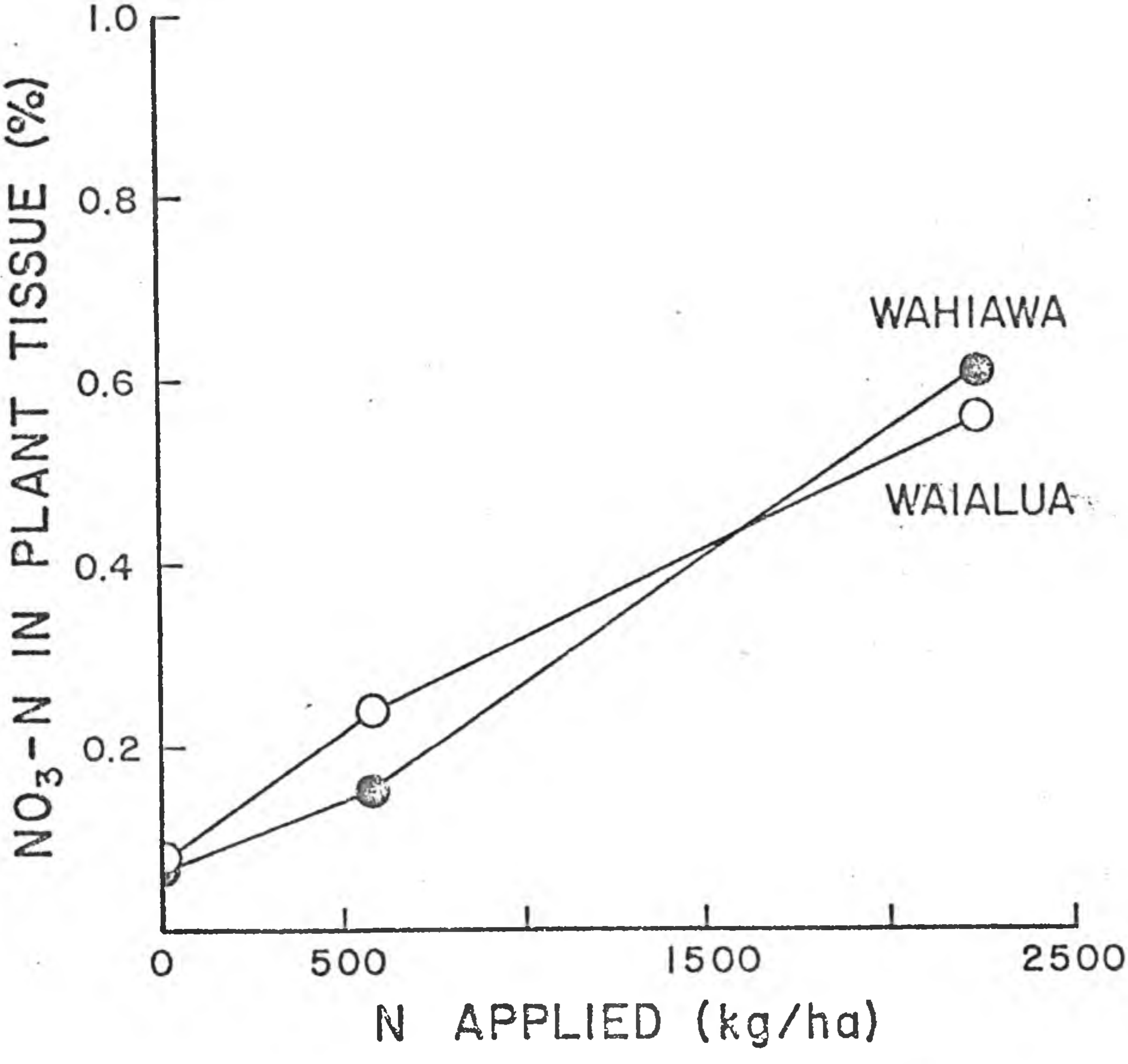

Figure 4. The NO_3-N content of sudangrass (ratoon crop) grown in the Waialua and the Wahiawa soils at different levels of N fertilization.

the difference in dilution effect between the two crops. The other factor that may be considered is the effect of other nutrients. In the ratoon crop, no further addition of other nutrients was made. A major portion of the nutrients applied at the beginning of the growing period would have been depleted by the first crop. Thus, the ratoon crop had a lower amount of "other-than-N" nutrients during the growing period than the plant crop. Several papers (Cantliffe, 1974; Smith and Clark, 1968; Barker and Tucker, 1971) in the past reported that the status of other nutrients have an influence on nitrate accumulation. Thus, the difference in nitrate - N content between the two sudangrass crops could have been due to this effect.

The total - N content of the ratoon crop was significantly affected by N fertilization (Table 17). The total - N content increased with an increase in N application (Table 15). The nitrate - N to total - N ratio also increased with an increase in N application.

C. Effects of Cattle Manure on Nitrate Accumulation in Plants

The practice of high rates of manure application to the soil results in an addition of a large amount of N (Weeks, 1972; Murphy, 1972). This N originally in a reduced form will eventually be nitrified to nitrate in the soil. This converted nitrate can pose a threat to animal and human health by accumulating in forage or by contaminating underground water. This experiment attempted to evaluate the effect of heavy manure application on nitrate accumulation in plants which are known to be high nitrate accumulators.

1. Nitrate - N Content

The nitrate - N content of sudangrass, spinach and spiny

Table 17. Analysis of variance of the total-N concentration of sudangrass ratoon-crop grown in two soils with three rates of N fertilization

Source of variation	DF	Mean square
Blocks	2	
Soil(S)	1	0.000939
Nitrogen(N)	2	2.631372**
S X N	2	0.0020354
Error	10	0.0280708

**Significance at the 0.01 level of probability

Table 18. Analysis of variance of the dry-matter yield of sudangrass ratoon-crop grown in two soils with three rates of N fertilization

Source of variation	DF	Mean square
Blocks	2	
Nitrogen(N)	2	3590.729**
Soil(S)	1	35.280
S X N	2	3.7065
Error	10	69.3359

** Significance at the 0.01 level of probability amaranth as affected by varying rates of manure application are presented in Tables 19 and 20. Nitrate accumulation was significantly affected by plant species (Table 21). The difference in accumulation between spinach and spiny amaranth was not significant. These two species differed in accumulation from that in sudangrass. These results were similar to those obtained in the earlier experiment with inorganic - N fertilizer. Also, as in the earlier experiment the difference in the nitrate content among the species was generally more marked at the higher levels (100 and 200 T/A) than at the lower level (to T/A) of manure application.

The accumulation of nitrate in all three species was directly related to manure application. The effect of manure application on the nitrate - N content in all the species studied was significant (Table 21). This was because the application of manure resulted in the addition of N to the soil (Weeks, 1972; Murphy, 1972). A large portion of the total - N in the manure is in the reduced form with varying degrees of stability (Salter and Schollenberger, 1939) and the amount of nitrate - N present is negligible (Miner, 1966). However, the application of manure not only increases the soil total - N content but also in several reports (Mathers and Stewart, 1970; Weeks, 1972) it was shown to increase the soil nitrate - N content. This is because under field conditions, a major portion of the N applied in the manure is nitrified to nitrate - N by microbial activity. Thus, the soil nitrate - N content is increased and available for absorption by plants. The tendency for nitrate accumulation to increase in plants with manure application was reported by several earlier workers (Hanway and Englehorn, 1958; Pratt, 1972; Weeks, 1972).

Table 19. The NO_3-N and total-N of sudangrass, spinach and spiny amaranth grown in the Wahiawa soil at different levels of manure application

Species	Manure applied	NO_3-N	Total-N	NO_3-N/Total-N
	T/A	%		
Sudangrass	0	0.077^{a}	0.53^{a}	14.53
	50	0.101^{a}	1.44^{b}	7.02
	100	0.380^{b}	2.31^{c}	16.45
	200	0.733^{c}	3.10^{d}	23.64
$\bar{x}$	-	0.323	1.84	-
Spinach	0	0.064^{a}	1.41^{a}	4.54
	50	0.127^{a}	2.17^{b}	5.85
	100	0.180^{a}	2.26^{b}	7.96
	200	1.500^{b}	4.48^{c}	33.48
$\bar{x}$	-	0.468	2.37	-
Spiny amaranth	0	0.052^{a}	1.53^{a}	3.40
	50	0.183^{a}	2.40^{b}	7.62
	100	0.297^{a}	2.84^{c}	10.21
	200	1.266^{b}	4.06^{d}	31.18
$\bar{x}$	-	0.450	2.71	-

Means for a single species followed by different letters are significantly different at the 0.05 level.

Table 20. The NO_3-N and total-N content of sudangrass, spinach and spiny amaranth grown in the Waialua soil at different levels of manure application

Species	Manure applied	NO_3-N	Total-N	NO_3-N/Total-N
	T/A	%		
Sudangrass	0	0.127^{a}	1.24^{a}	10.28
	50	0.270^{ab}	1.86^{a}	14.52
	100	0.440^{bc}	1.89^{a}	23.28
	200	0.580^{c}	2.58^{b}	22.52
$\bar{x}$	-	0.354	1.89	-
Spinach	0	0.087^{a}	1.92^{a}	4.53
	50	$0,617^{b}$	2.89^{b}	21.35
	100	1.120^{c}	2.73^{b}	41.02
	200	1.350^{c}	4.03^{c}	26.30
$\bar{x}$	-	0.794	2.41^{b}	-
Spiny amaranth	0	0.071^{a}	1.85^{a}	3.84
	50	0.293^{b}	2.86^{b}	10.25
	100	0.913^{c}	3.08^{b}	29.65
	200	1.567^{d}	4.54^{c}	34.52
$\bar{x}$	-	0.711	3.08	-

Means for a single species followed by different letters are significantly different at the 0.05 level.

Table 21. Analysis of variance of the nitrate-N concentration of three plant species grown in two soils with four rates of manure application

Source of variation	DF	Mean square
Blocks	2	
Species(Sp)	2	0.596355**
Soil(S)	1	0.782708**
Manure(M)	3	4.0483013**
S X Sp	2	5.08740*
Sp X M	6	0.2502085**
S X M	3	0.028331
S X Sp X M	6	0.1127709**
Error	46	0.0277708

*Significance at the 0.05 level of probability

**Significance at the 0.01 level of probability

In sudangrass the nitrate accumulation response curve differs between the two soils (Figure 5). In plants grown in the Wahiawa soil the nitrate - N content reached the toxic level of 0.15% nitrate - N (Ryan, 1972; Murphy and Smith, 1967) only when manure was applied at more than the 50 T/A rate. An application of manure below this 50 T/A rate would not pose any problem of accumulating a toxic level of nitrate - N in sudangrass grown in this soil. However, in sudangrass grown in the Waialua soil the toxic level of nitrate - N in plants was found to occur even at a manure application of below 50 T/A. The highest nitrate - N content in sudangrass was found to be 0.733% in plants grown in the Wahiawa soil receiving 200 T/A of manure. Pratt et al. (1972) reported that the nitrate - N of sudangrass forage exceeded 0.22%, which they considered the toxic level, when 40 to 80 T/A of manure was applied. Murphy et al. (1972) reported that in corn, the maximum amount of nitrate - N accumulated was found to be only 700 ppm (0.07%), which is well below the toxic level, in spite of the highest level of manure application being 700 T/A.

The trend by which nitrate was accumulated in spinach differed in the two soils in which the plant was grown (Figure 6). The nitrate - N content of plants grown in the Waialua soil rose sharply up to the application of 100 T/A of manure; the curve then tended to flatten-off with a further increase in manure application. The increase in nitrate - N content of spinach grown in the Wahiawa soil followed a different trend. The increase was very low up to the 100 T/A rate and rose sharply with an application of manure above the 100 T/A rate. The highest value of nitrate - N accumulated was found to be 50% in plants grown in the Wahiawa soil at the 200 T/A application (Table 19).

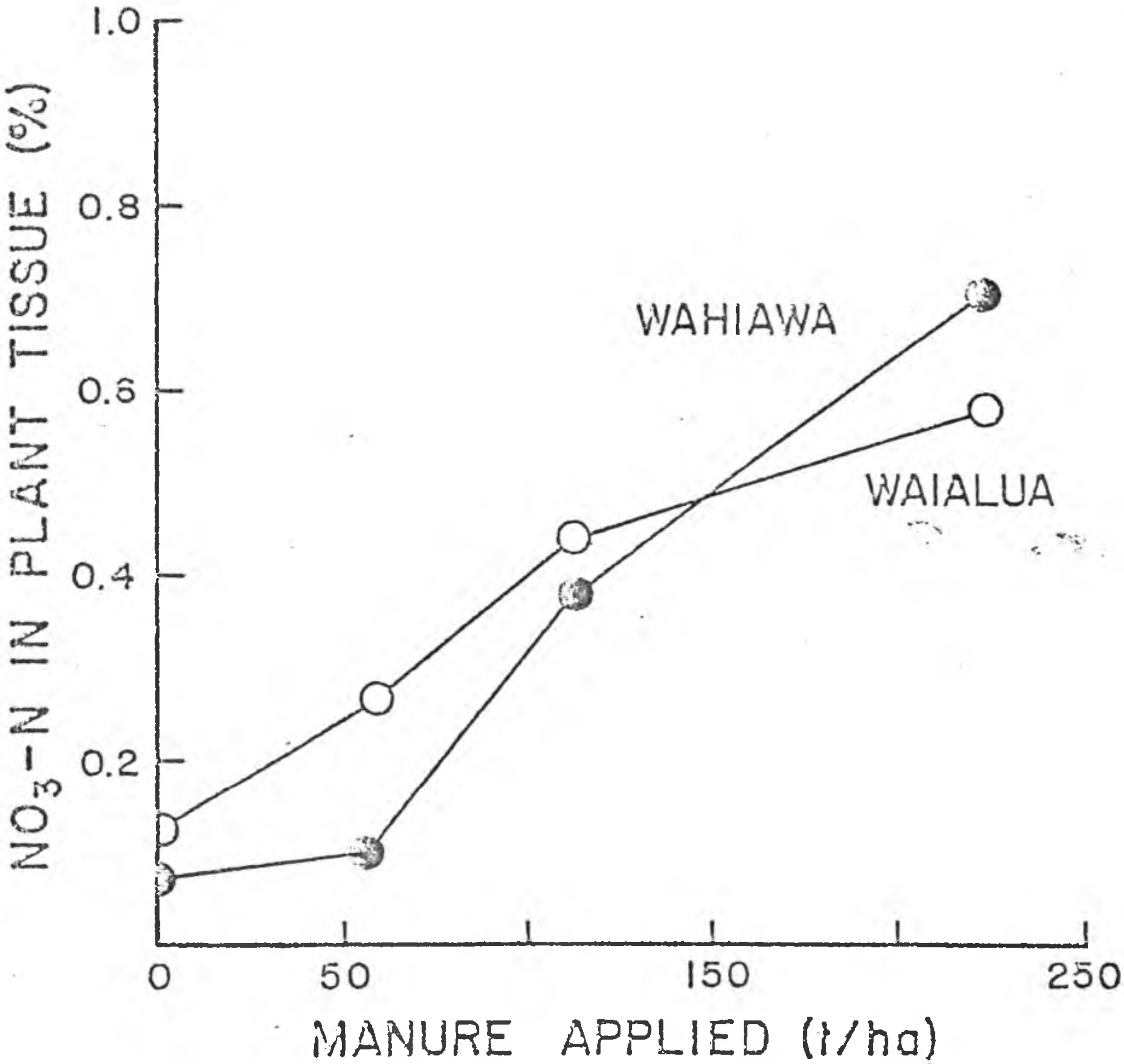

Figure 5. The NO_3-N content of sudangrass grown in the Wahiawa and the Waialua soils as affected by manure application.

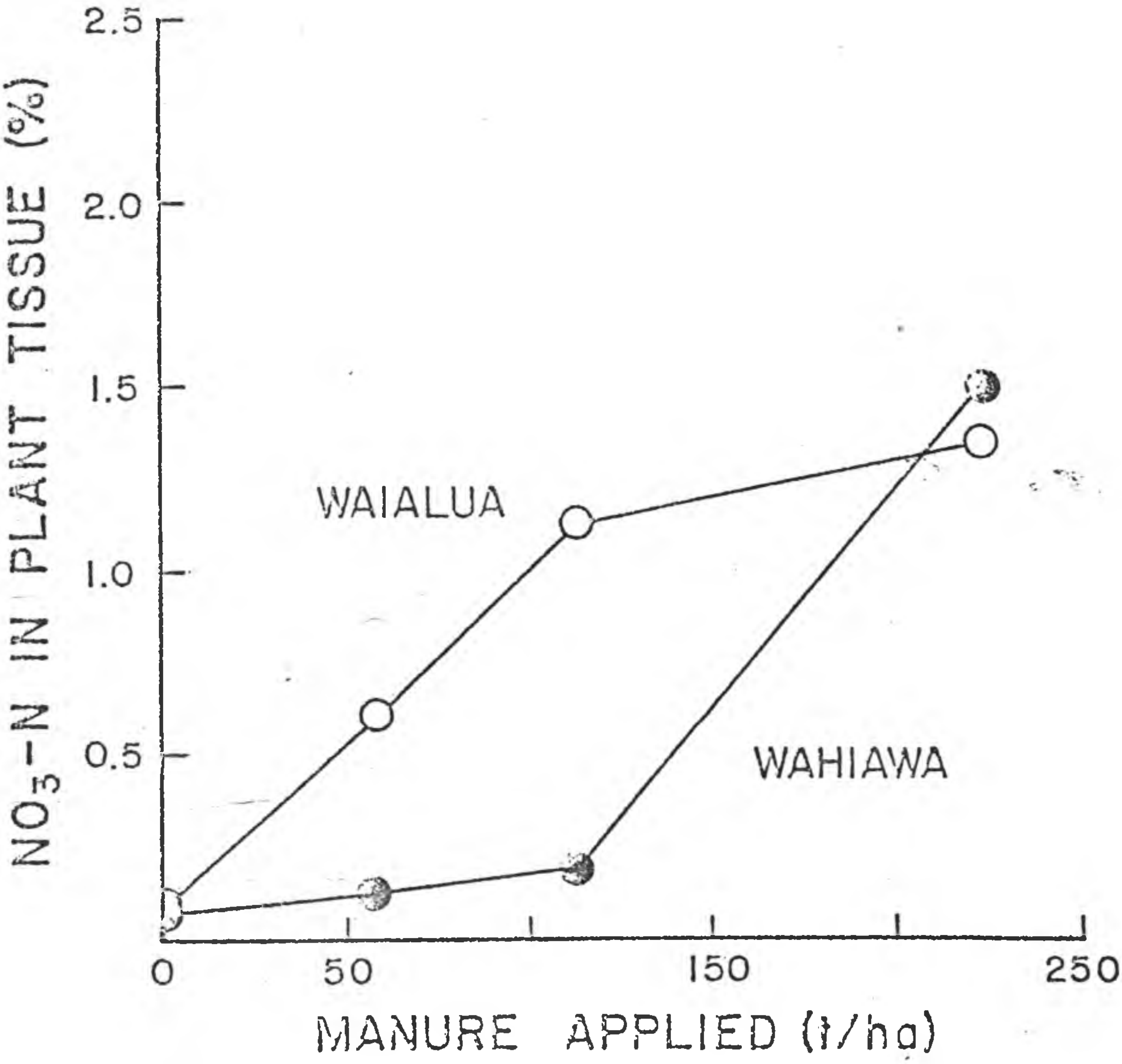

Figure 6. The NO_3-N content of spinach grown in the Wahiawa and the Waialua soils as affected by manure application.

However, it should be pointed out that the spinach plants grown in the Wahiawa soil receiving a manure application of 100 T/A were growing abnormally at the early stage of growth but recovered later as the plants matured (see note on page 21). This factor should be considered for it may have been responsible for the difference in the trends by which nitrate was accumulated in the two soils.

Simon et al. (1966) estimated that processed spinach for baby food should not exceed 300 ppm nitrate (67 ppm nitrate - N; authors note: this is presumably on a fresh weight basis). If this level is considered as the toxic level for consumption by human babies, then the nitrate - N in all the spinach in this study would exceed the standard. However, Brown and Smith (1967) estimated that the tolerance level might be higher and, also, a major portion of the nitrate can be extracted through cooking. A potential danger for nitrate poisoning could exist if the nitrate - N content of the spinach is high enough and if a sufficient quantity is ingested, particularly if it is uncooked.

The nitrate - N content of spiny amaranth was greatly influenced by the level of manure applied. Nitrate - N accumulation differed between the plants grown in the Waialua and Wahiawa soils (Figure 7). The plants grown in the Waialua soil showed a higher nitrate accumulation than those grown in the Wahiawa soil. The nitrate - N content curve did not seem to flatten-off, even with the application of 200 T/A of manure, indicating that more nitrate could have been accumulated at above the 200 T/A application. The nitrate - N content reached as high as 1.567% in plants grown in the Waialua soil receiving 200 T/A

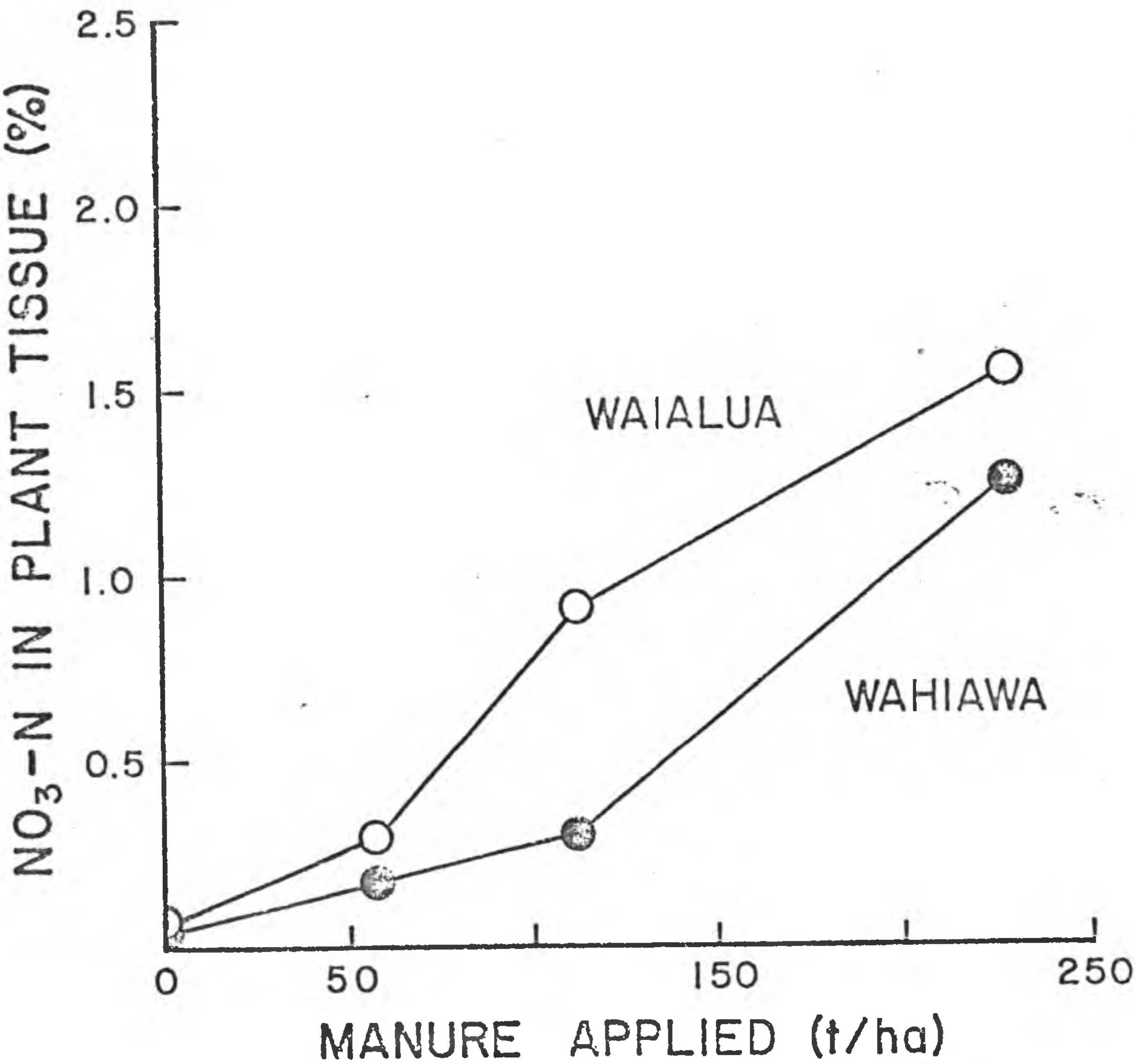

Figure 7. The NO_3-N content of spiny amaranth grown in the Wahiawa and the Waialua soils as affected by manure application.

of manure (Table 20). The tendency of this species of weed to accumulate a high amount of nitrate should be of concern to livestock operators. This species of weed is very common and usually is found in abundance in pasture lands; thus, it has a great chance of being ingested by animals through grazing or through inclusion in the animal feed. Previous mention was made by Bradley (1940) that cattle were killed after feeding on hay that contained this weed.

In all three species the amount of nitrate - N accumulated was affected by soil types. The nitrate - N levels within each species as influenced by soil types and by rate of manure application are shown in Figures 5, 6 and 7. The nitrate - N accumulated by plants grown in the Waialua soil was significantly higher ($p \leq 0.01$) than the nitrate - N accumulated by plants grown in Wahiawa soil (Table 21). In two (sudangrass and spinach) of the three species grown in the Waialua soil, the accumulation of nitrate seemed to have started to level off when manure was applied between 100 to 200 T/A. In corn forage it was reported by Murphy et al. (1972) that the nitrate - N content tends to reach a peak at about 300 to 400 T/A rates of manure applied; however, the manure used in their study had a total - N content of 1.04% as compared to 4.28% used in this study (see Table 3).

The difference in nitrate accumulation in plants grown in the two soils perhaps can be attributed to a large extent to the difference in the amount of total - N present in the nitrate form in the two soils. A major portion of the total - N present in manure is in a reduced form (Salter and Schollenberger, 1939). The nitrate content in the manure was very small (see Table 3). Thus, the amount of nitrate present in the soil after manure application would be a function of the soil nitrification rate. If the initial nitrate - N content of the soil is any indication of the nitrification rate of the soil, then the Waialua soil can be said to have a higher rate than the Wahiawa soil because the former had a much higher nitrate - N content than the latter (see Table 1). Also, according to Briones (1969) the soil total - N content and nitrification rate positively correlated; the data in Table 1 indicate that the Waialua soil has a higher total - N content than the Wahiawa soil. These two soils were also found to differ in their rate of N - mineralization (Briones, 1969); she reported that the Waialua soil has a higher mineralization rate than the Wahiawa soil. Based on these evidences, it can be concluded that the difference in nitrate accumulated in plants grown in the two soils can be attributed to the difference in the amount of total - N present in the nitrate form after nitrification had taken place with manure application. The plants grown in the Waialua soil has a higher nitrate content at comparable manure levels because of this difference.

As was discussed earlier in the inorganic - N experiment, these two soils differ in "texture". The difference in nitrate accumulated in plants grown in these two soils probably can be attributed to this "textural" difference. It has been shown in an earlier report (Lovelace, 1968) that soil texture influence nitrate accumulation in plants. In this experiment, in several instances, leaching could not be avoided. This resulted in a differential loss of soil nitrate - N between the two soils. More nitrate would be expected to be leached out of the Wahiawa soil than the Waialua soil due to the differences in physical characteristics discussed earlier. This difference perhaps could account for the difference in nitrate accumulation in plants grown in the two soils in addition to the difference in nitrification rates discussed above.

2. Total - N

The total - N contents of sudangrass, spinach and spiny amaranth as influenced by varying rates of manure application are presented in Tables 19 and 20. The total - N content was found to be directly related to manure application. The content increased along with the nitrate - N with an increase in manure application; however, the trend differed. The total - N content in all three species did not seem to level off as did the nitrate - N content, even with the highest manure application of 200 T/A.

The total - N content of the plants in this study was significantly affected by manure application, plant species and soil types (Table 22). These factors are similar to those affecting nitrate accumulation. There was only a small difference in total - N content at comparable manure application rates in spinach and spiny amaranth; however, the difference in content between these two species and sudangrass was very great (Tables 19 and 20).

To evaluate the rate of nitrate assimilation with increasing manure application, the nitrate - to total - N ratio was calculated. These ratios are presented in Tables 19 and 20. In all treatments, with the exception of sudangrass grown in the Wahiawa soil and spinach grown in the Waialua soil, the ratio increased with an increase in manure application. This indicates that the rate of nitrate assimilation per unit nitrate absorbed was reduced with increasing

Table 22. Analysis of variance of the total-N concentration of three plant species grown in two soils with four rates of manure application

Source of variance	DF	Mean square
Blocks	2	
Species(Sp)	2	7.05858**
Manure(M)	3	17.997353**
Soil(S)	1	0.873380**
S X M	3	0.3728433*
S X Sp	2	0.158225
Sp X M	6	0.4257716**
Sp X N X M	6	0.1855443
Error	46	0.0914776

*Significance at the 0.05 level of probability

**Significance at the 0.01 level of probability

manure application. This trend was also observed in the inorganic experiment and also was reported by several earlier workers (Whitehead, 1948; Smith and Clark, 1968; Flynn, 1957).

3. Yield and Plant Growth

The effect of varying rates of manure application on plant dry matter yield are given in Table 23. The effects of manure applications, soil type and plant species were found to be significant (Table 24). In all three species the dry matter yield did not increase with the application of manure above 50 T/A. Weeks et al. (1972) reported that in corn no economic advantage could be obtained from manure application in excess of 20 T/A. Application of more than 50 T/A also was found to cause poor germination, especially in spinach and spiny amaranth, while sudangrass germination was less affected. The effect of manure on germination was reported by Adriano et al. (1973), who also found that sudangrass germination was less affected than that of spinach.

Application rates in the range of 100 to 200 T/A caused abnormal growth in the plants studied. In all three species growth was slow and the plants were stunted. Sudangrass was more severely affected than the other two species; the plants exhibited symptoms of water stress with brown discoloration on the tips of young leaves and in severe cases the leaf tips stuck together. This effect was suspected to be caused by salt injury. The soil E_c reading at different levels of manure application was made on a 1:1 soil to water extract. The data in Table 25 show the effect of manure application on the E_c reading. The E_c reading of the soil was found to increase with an increase in manure application. Thus, the decline in dry matter yield in all

Table 23. The vegetative yield of sudangrass, spinach and spiny amaranth as affected by manure application

Species	Manure applied	Dry-matter yield	
		Wahiawa	Waialua
Sudangrass	0	35.36[ab]	52.40[a]
	50	53.93[b]	79.99[a]
	100	34.65[ab]	68.00[a]
	200	22.66[a]	52.99[a]
Spinach	0	8.80[a]	34.36[a]
	50	32.19[a]	40.79[a]
	100	20.77[a]	28.51[a]
	200	32.30[a]	34.94[a]
Spiny amaranth	0	10.69[a]	27.27[a]
	50	35.48[a]	37.26[a]
	100	23.00[a]	28.36[a]
	200	25.32[a]	33.54[a]

Means for a single species followed by different letters are significantly different at the 0.05 level.

Table 24. Analysis of variance of the dry-matter yield of three plant species grown in two soils with four rates of manure application

Source of variation	DF	Mean square
Blocks	2	
Manure(M)	3	1100.2103
Soil(S)	1	4197.264**
Species(Sp)	2	3760.964**
S X M	3	48.457
S X Sp	2	601.454
Sp X N	6	234.4668
S X Sp X M	6	118.4675
Error	46	268.72447

*Significance at the 0.05 level of probability

**Significance at the 0.01 level of probability

Table 25. Soil conductivity at varying levels of manure application

Manure applied	Conductivity	
	Wahiawa	Waialua
T/A	millimhos/cm($EcX10^3$)	
0	0.8030	0.6155
50	2.655	3.845
200	8.055	8.500

Ec reading of soils with 100 T/A application were not determined because soils were accidently removed.

three species could be attributed to salt injury caused by increasing salt concentrations with increasing manure applications. Sudangrass was most severely affected because it has a poor tolerance to excess salt as compared to spinach (Richards, 1969). The salinity level at which a 50% yield decrease will be expected for sudangrass is at an E_c value of about 9 mmhos/cm and at about 11 mmhos/cm for spinach, these values are based on saturation extracts and they will be much lower if they are based on 1:1 soil to water ratio extracts as in this study (Richards, 1969).

SUMMARY AND CONCLUSIONS

The study consisted of two parts: a survey of some common weeds and forage crops from different locations for their nitrate - N content and a greenhouse experiment to study the effects of (1) inorganic - N fertilizer and (2) cattle manure on nitrate accumulation in plants.

The nitrate content of some common weeds collected from the University of Hawaii Manoa Campus was analyzed. The nitrate - N content ranged from 220 to 4,150 ppm. The nitrate - N was found to vary with plant species. The spiny amaranth was found to be the highest nitrate accumulator (4,150 ppm of nitrate - N). Other plant species that were found to have a high nitrate content were bristly foxtail, pigweed and guineagrass. The rest of the weeds collected seemed to have a low nitrate - N content.

The second group of plant samples were collected from the Foremost Dairy Farm, Waimanalo, Oahu. The plant samples were collected from three different plots that had different levels of manure application. These plots were: (1) FD - V (an uncultivated plot that did not receive any manure application), (2) FD - 6 and FD - 7 (these plots received a heavy application of manure slurry every six weeks) and (3) FD - M (this plot received a moderatly low amount of manure application).

The nitrate - N content of samples collected from the uncultivated plot (FD - V) was found to range from 460 to 4,900 ppm. Spiny amaranth was the highest nitrate accumulator with a nitrate - N content of 4,900 ppm. Other species that were high in nitrate - N content were pigweed, paragrass and wiregrass.

The nitrate - N content in the plant samples collected from the FD - 6 and FD - 7 plots were found to be much higher than in those collected from the FD - V plot. The two forage crops, sudax and paragrass, grown in these fields were found to have a nitrate - N content of 8,000 ppm and 4,000 ppm respectively. These values were much higher than the "safe" level in forage crops for livestock consumption. Spiny amaranth was found to be the highest nitrate accumulator with a nitrate - N content of 11,500 ppm, which is three times as much in content as in those samples collected from the untreated plot (FD - V). In most weeds, including those which are normally low nitrate accumulators, the nitrate - N content was above 1,000 ppm.

The nitrate - N content in the samples collected from the "mauka" field (FD - M) was higher than in the samples collected from the uncultivated field (FD - V) but lower than in the samples collected from the field receiving heavy applications of manure slurry (FD - 6 and FD - 7). The forage crop, guineagrass, growing in this "mauka" field was found to have a nitrate - N content of 1,500 ppm. The spiny amaranth was found to have a nitrate - N content of 4,000 ppm. Other species of weeds collected from this field were found to have a higher nitrate - N content than those from the virgin plot.

Two species of plants, spiny amaranth and sudax, from FD - 7 were analyzed for nitrate - N at different stages of maturity. In sudax the nitrate - N content was higher in the younger plants than in the older plants, while in spiny amaranth the nitrate - N content was higher in the older plants than in the younger plants. Variation of nitrate - N content in spiny amaranth as affected by different parts of the plant was studied. It was found that the nitrate - N content in the stem was much higher than in the leaf.

The effects of inorganic - N fertilizer on nitrate accumulation, in sudangrass, spinach and spiny amaranth were studied. The plants were grown in the Wahiawa and the Waialua soils with 0, 500 and 2,000 lb N/A rates. The effects of plant species, soil types and N rates were found to be significant (p 0.05). Spiny amaranth and spinach generally accumulated a much higher amount of nitrate than sudangrass. Nitrate accumulation was significantly higher in plants grown in the Waialua soil than in plants grown in the Wahiawa soil.

Generally, the increase in the nitrate - N content in the three species with an application of 500 lb N/A rate was small. In sudangrass the increase was not significant and the nitrate - N content did not exceed the toxic level for forage at this N level. However, with an application of 2,000 lb N/A rate, the nitrate - N content in all three species was significantly increased. In sudangrass the nitrate - N content was much higher than the suggested toxic level, while in spinach and spiny amaranth the nitrate - N content was higher than 1%.

The total - N content of the three species studied was significantly affected by N application, plant species and soil types. These factors were similar to those affecting nitrate accumulation.

The ratio of nitrate to total - N for each treatment was calculated. In general, this ratio increased with an increase in N application.

In all three species N application produced a significant ($p \leq 0.05$) increase in yield; however, this increase tended to level off with an N application above 500 lb N/A.

In the sudangrass ratoon crop, nitrate accumulation was affected only by N levels. The effect of soil types was not significant. An application of 500 lb N/A resulted in an increase in nitrate - N content above the toxic level. However, the increase in dry - matter was only significant when N was applied at the 2,000 lb N/A rate. The total - N content was significantly affected by N levels and was also affected by soil types. The nitrate - N to total - N ratio increased with N application.

The effects of dairy manure application on nitrate accumulation in sudangrass, spinach and spiny amaranth were studied. The plants were grown in the Wahiawa and the Waialua soils with 0, 50, 100 and 200 T/A of manure applications. The nitrate accumulation was significantly affected by rates of manure application, soil types and plant species. Generally, spinach and spiny amaranth accumulated more nitrate than sudangrass. Nitrate accumulation was significantly higher in plants grown in the Waialua soil than in plants grown in the Wahiawa soil. The shape of the nitrate response curve differed between the two soils. In two (sudangrass and spinach) of the three species grown in the Waialua soil, the accumulation of nitrate seemed to have started to level - off when manure was applied between 100 to 200 T/A. However, the nitrate - N content response curve in the Wahiawa soil did not seem to level - off at these levels. In sudangrass grown in the Wahiawa soil, the nitrate - N content reached the toxic level only when manure was applied at more than the 50 T/A rate; however, in plants grown in the Waialua soil the toxic level was reached at a lower rate.

The total - N content of the three species studied was significantly affected by rates of manure application, soil types and plant species.

These factors are similar in effects to those affecting nitrate accumulation. The ratio of the nitrate - N to the total - N for each treatment was calculated. This ratio generally increased with an increase in manure application. The dry matter yield in all three species was significantly increased up to the 50 T/A rate of manure application; an application above this rate resulted in a yield reduction. This reduction was attributed to a high salt concentration. Sudangrass showed very severe salt injury symptoms when manure application was higher than 50 T/A.

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